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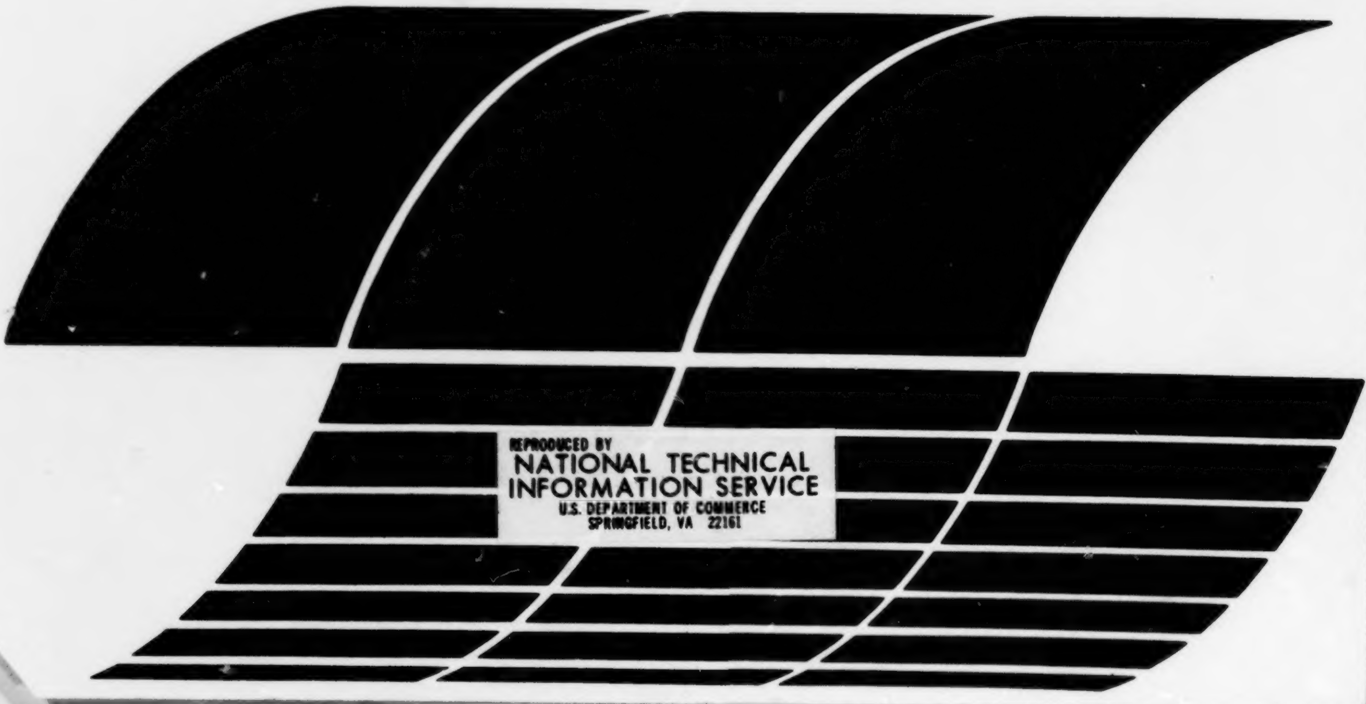

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Experimental studies of the effects of Prudhoe Bay crude oil on the recovery of intertidal infauna and epifauna were conducted in the Strait of Juan de Fuca region of Washington State. The studies experimentally evaluated the effect of oil treatment, site, substrate type, season, and tide level on the composition, density, and species richness of organisms colonizing substrates which were initially free of organisms. Significant differences for some biological parameters were demonstrated for each of the types of treatment contrast (site, substrate type, season, tide level, and oil). Significant biological effects were demonstrated to be due to oil treatments for 70% of 56 biological parameters evaluated in detail.

Full recovery following contamination with oil was predicted for sediment-borne infauna based on oil retention time and recovery of infauna in unoiled sediments. Predicted full recovery for sand habitats at Sequim Bay and Protection Island was 31 months following an initial oil treatment of 1,800 ppm. Predicted full recovery for commercial clam bed habitat was 46 months following an initial oil treatment of 2,500 ppm. "Best" and "worst" cases for chemical recovery of oiled concrete substrates were 3 and 13 months.

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FINAL REPORT

by

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by

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FOREWORD

An anticipated increase in oil tanker traffic and proposals for construction of subsea pipelines in the Strait of Juan de Fuca and northern Puget Sound regions of Washington State are foreseen as part of the national energy development plans. These activities increase the opportunity for spillage of crude oil into the marine ecosystems of the region. The U.S. Environmental Protection Agency has supported studies dealing with biological characterizations, physical oceanography, trajectory modeling, pollutant monitoring, and fate and effects of oil in the region. These studies are being administered by NOAA's Marine Ecosystems Analysis (MESA) Puget Sound Project Office. The research reported here deals with recovery of intertidal and shallow subtidal communities in experimental habitats contaminated with Prudhoe Bay crude oil. The studies make comparisons in rate of recovery by communities in experimental coarse and fine sand habitat and hard substrate habitat, and a commercial clam bed. They examine the role of vertical distribution of habitat in the tidal zone, site, type of substrate, season, and duration for recovery in field experiments.

ABSTRACT

Experimental studies of the effects of Prudhoe Bay crude oil on the recovery of intertidal infauna and epifauna were conducted in the Strait of Juan de Fuca region of Washington State. The studies experimentally evaluated the effect of oil treatment, site, substrate type, season, and tide level on the composition, density, and species richness of organisms colonizing substrates which were initially free of organisms. Significant differences for some biological parameters were demonstrated for each of the types of treatment contrasted (site, substrate type, season, tide level, and oil). Significant biological effects were demonstrated to be due to oil treatments for 70% of 56 biological parameters evaluated in detail.

Full recovery following contamination with oil was predicted for sediment-borne infauna based on oil retention time and recovery of infauna in unoiled sediments. Full recovery for epifauna on concrete substrates could not be predicted from these studies because of the longer-lived nature of dominant species and differing assumptions about what constitutes full recovery. Predicted full recovery for sand habitats at Sequim Bay and Protection Island was 31 months following an initial oil treatment of 1,800 ppm. Predicted full recovery for a commercial clam bed habitat was 46 months following an initial oil treatment of 2,500 ppm. Density of the principal species of interest on this clam bed (Protothaca staminea) was significantly altered by the oil treatment during the first recruitment season. Because of the longer-lived (compared to the general infauna community) nature of this species, it was predicted that effects on recovery of the species may extend somewhat beyond that for the general infaunal community. "Best" and "worst" cases for chemical recovery of oiled concrete substrates were three and 13 months.

Effects from oiling on recovery is strongly related to feeding type of infauna and epifauna but the influence is different depending on habitat. For the sand habitats, detritivorous and herbivorous species were almost universally influenced by the oiling. Carnivorous species were about evenly divided in their response to the oiling and, with one exception, no significant effect was seen on the recovery of a suspension feeder. For the commercial clam bed, herbivores and suspension feeders were at least as sensitive to the oil treatment as detritivores. For the concrete habitat, detritivores were not sensitive to the effect of oil treatment but herbivores and suspension-feeders were highly sensitive. Based on adjunct MESA studies of trophic relationships, it appears that the severity of the influence on recovery of species in this study could be expected to have a deleterious effect on important fish populations, and that the effect would extend somewhat beyond the 15-month period studied in individual experiments in this program.

Retention of oil differed depending on substrate type, tidal height, and initial concentration. Concrete substrates lost oil much more quickly than sediments. Oil was retained longer at higher tide levels than at lower tide levels. Proportionally more oil was retained in sediments initially treated with higher concentrations of oil.

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SECTION 1

INTRODUCTION

The Strait of Juan de Fuca and northern Puget Sound regions of Washington State represent some of the nation's finest inland marine habitat. Historically, the fishery and shell fishery have been prime sources of industry and recreation. Noncommercial organisms in shoreline habitats represent a source of enjoyment for residents of the region and give impetus to a flourishing tourist industry. Deep water in the channels of the Strait and the natural harbors provide a basis for a large shipping industry. Four major oil refineries in the north Puget Sound region have had the capacity to provide petroleum products sufficient for the state's needs. Until the OPEC embargo in 1973, the crude oil for these refineries was supplied mainly by pipeline from Canadian oil fields. Since that time the state's refineries have relied on tanker transport of crude oil, first from foreign sources and, since completion of the trans-Alaska pipeline, increasingly from Alaskan sources. At the present time, the Strait of Juan de Fuca is being considered as a throughpoint for oil to be shipped from Alaska to mid-western markets. Such use would result in an order of magnitude increase in the amount of crude oil shipped in the marine waters of the Strait of Juan de Fuca as well as to increase the risk from spillage at off-loading facilities. There is public concern about potential environmental damage resulting from spillage of crude oil into marine habitats.

Against the foregoing background, the U.S. Environmental Protection Agency, in studies administered by the Marine Ecosystems Analysis Project Puget Sound, initiated a broad range of marine environmental studies in the north Puget Sound region. The major portion of these studies was aimed at gaining an inventory of the marine biota in the Strait of Juan de Fuca and the northern Puget Sound region, and describing physical transport processes which could aid in predicting movement of spilled petroleum in the Strait of Juan de Fuca-northern Puget Sound region. As an adjunct to biological inventories and studies of physical processes, the present studies were undertaken to experimentally measure potential recovery rates of organisms and communities within especially vulnerable habitats should they become impacted by petroleum. While direct studies of the effects of petroleum on specific organisms and the biological fate of petroleum have been and will continue to be addressed by other agencies, the studies reported here involve the experimental application of petroleum to shoreline habitat units to allow comparative evaluation of recovery under oiled and unoiled conditions.

Shoreline habitats are most vulnerable to spilled oil for at least two major reasons. First, at the interface of water and land, the non-soluble, floating oil residue comes in direct contact with the substrate. Because of changing tide and wave action, this material can be mixed with the substrate in the case of fine, movable materials (mud, fine and coarse sand, pea gravel) and repeatedly applied to the surface in the case of rock. The mechanisms of effect on marine organisms are not necessarily related to the toxicity of the petroleum but rather to such things as physical smothering (rendering substrate unsuitable for settlement) or interference with behavior processes. A second factor resulting in the high vulnerability of shoreline habitats to oil relates to the heterogeneity of the habitats. Unlike open water or the relatively smooth expanses of some bottom habitats, the shore consists of myriad irregularities formed by cracks and pools and mixes of substrate sizes. This heterogeneity allows isolated concentrations of petroleum in both water and substrate and the consequent exposure of populations for extended periods.

The habitat heterogeneity that contributes to the vulnerability of specific shore habitats also contributes substantially to a high amount of variation in the population spatial distribution within these habitats. In studies of the MESA Puget Sound Project which had the goal of inventorying shore communities (Nyblade, 1978; Webber 1979), difficulties in measuring community variables due to high spatial variation were mitigated by a classification of shoreline habitats into types relating to the predominant substrate. Nowhere do the types exist in a "pure" form. Each of the types contains sub-parts that are clearly of each of the other types. The goal of the present studies has been to test hypotheses about the influence of Prudhoe Bay crude oil on the recovery of intertidal communities related to type. To achieve this goal, the inherent heterogeneity has dictated an experimental approach using units of "pure" type.

Three types of habitat were chosen for recovery studies: (1) concrete; (2) sand; and (3) a commercial clam bed. Even in the "pure" form imposed by the experimental approach used here, the types differ markedly in their heterogeneity. The reasons for selection of the specific types also differed. Rock is the predominant type in the Strait of Juan de Fuca and San Juan Island portions of the northern Puget Sound region. Its expanse alone gives sufficient reason for high priority in study of its recovery potential. In addition, based on inventory studies of the Strait of Juan de Fuca (Nyblade, 1978; 1979), rock habitat is perhaps the most highly diverse and productive in the region. Unfortunately, from the standpoint of hypothesis testing studies, rock also exhibits the highest degree of spatial heterogeneity in its populations. Estimates of numbers of individuals for a population at a given site typically exhibit a percent coefficient of variation from 150 to several hundred percent of the mean number. In these studies concrete was used to represent rock. Sand habitats are also quite abundant in more or less "pure" form in the northern Puget Sound region. Coarse, mobile sand habitats are among the lowest in productivity in the Strait of Juan de Fuca region (Nyblade, 1979). However, where sand is stable and finer components are contained, the sand habitat is extremely important for fish food organisms. The primary interest in sand habitat in this study stems from the presence of food organisms for bottom-feeding fishes contained therein. The habitat is considerably less diverse and productive overall than is rock. A highly attractive feature of sand habitat for experimental studies is that the spatial heterogeneity within units amenable for experimental study is perhaps at its lowest for intertidal habitat. The third type investigated here, a sand-pebble mixed habitat associated with a commercial clam bed, was chosen primarily for its functional role. Commercial shell fisheries are an important industry to the region. The substrates are sometimes "managed" in the sense that fine, highly organic materials are stabilized with coarse sand and pebble-size rock. Organism heterogeneity is less than for rock, but the diversity and productivity of these substrates exceeds that in pure sand habitat. The foregoing characteristics of the target habitats had direct bearing on the experiments planned and the specific objectives deemed feasible for this investigation.

In broad scale, the studies were divided into a number of tasks which encompass the specific objectives. The tasks relating to the sand habitat included:

A. Provide a time-series survey of species composition and total hydrocarbon concentration in experimentally prepared substrates.

B. Measure the effect from Prudhoe Bay crude oil on reseeding by early colonizers as related to site and substrate grain size during a late summer-early fall recruitment period.

C. Measure the effect from Prudhoe Bay crude oil on reseeding by later colonizers one year after initial colonization during the late summer-early fall recruitment period.

D. Measure at one experimental site the effect from Prudhoe Bay crude oil on reseeding by early colonizers during the late spring-early summer season.

E. Measure at one experimental site the effect from Prudhoe Bay crude oil on reseeding by early colonizers as related to tide height.

Tasks relating to the rock habitat included the following:

F. Investigate the suitability of some artifical hard substrates for attachment in the exposed rocky intertidal zone of the Strait of Juan de Fuca for future experimental recovery studies.

G. Investigate recovery of a rocky intertidal community in terms of larval reseeding rate for key species.

H. Investigate recovery of a rocky intertidal community in terms of mortality and removal of key species.

A single task dealt with the commercial clam bed sand-pebble habitat:

I. Investigate recovery of a commercial clam bed in terms of larval reseeding rate.

Tasks A through E, which dealt with recovery of infauna populations in fine and coarse sand habitats were reported in detail in an interim report on this project (Vanderhorst et al., 1980). Priority was given those tasks because: (1) background information (Vanderhorst et al., 1978) permitted the design of specific experiments; (2) the relative spatial homogenity for contained populations ensured cost effectiveness; and (3) important fish food organisms were contained in the habitat.

The objectives for Task A were: (1) to provide guidance in selection of an optimum concluding date for early colonization experiments in terms of hydrocarbon retention and colonization by infauna; (2) to provide a seasonal time series of species composition; and (3) to permit a continuing evaluation of sediment retention of oil between early and late colonization experiments.

The objectives for Task B were addressed in a single experiment. They were of three kinds. The first objective was to measure the effect of site, sediment source (particle sizes differed relating to source), and oil treatment on recovery (species density) for seven primary species in a valid hypothesis-testing framework. The experimental treatments were oil (two levels), site (two sites), sediment source (two sources); these were evaluated in a three-month late summer-fall seasonal framework. The second objective was to measure possible change in hydrocarbon concentration and composition from initiation to completion in the above experiment. The third objective was to measure the effect of the experimental treatments on recovery of all species (density, composition) in a descriptive statistical framework. For this latter objective, the same type of statistics were used as for the first objective, i.e., analyses of variance. However, because of the large numbers of species involved, we cannot be certain of the error probabilities.

The objectives for Task C were addressed in another independent experiment. They paralleled the objectives for Task B with three exceptions: (1) target densities and compositions were those occurring after 15 months of field exposure; (2) a single site was studied; and (3) the number of primary species was increased to ten from the original seven.

Task D and E objectives were addressed in a third independent experiment. Again, the specific objectives paralleled those for Task B. For these tasks, however, the experimental treatments were oil (two levels) and tide level (two levels). These treatments were evaluated in a three-month spring-summer seasonal framework.

The investigation of recovery of epifauna on the rock habitat (Tasks F, G, and H) had distinctly different objectives because of: (1) the severity of exposure conditions in which this type habitat normally occurs;

(2) the much higher degree of heterogeneity in spatial distribution for species as compared to the sand habitat; and (3) an expected much shorter retention time for oil on the substrate. Task F was an investigation of the feasibility of using various attachment methods for different artificial hard substrates. The results have been previously reported (Vanderhorst et al., 1980), and led to the use of the experimental approach reported for Tasks G and H.

The objectives of Task G were addressed in 12 independent experiments. Ten of these were conducted at a single site at monthly intervals (October 1979 through July 1980). Treatments were: oil (two levels) and tide height (two levels). The similar objectives for each of these experiments were to evaluate treatment effects on: (1) chemical characteristics immediately after oil treatment, and at five and 30-day intervals after field placement; (2) suitability of oiled substrates for colonization as indicated by the presence of marine larvae based on daily observations immediately following field placement; and (3) hypothesis tests for differences between treatment and control after 30 days field colonization for: major group densities (i.e., taxonomic groups = polychaetes, crustaceans, mollusks; trophic groups = herbivores, carnivores, suspension-feeders, detritivores; and species richness overall and for taxonomic groups. Two further experiments addressed these same objectives when an additional site was added as a treatment factor.

The objectives for Task H were based on a different premise than any of the other tasks, including sediment tasks, in these studies. Whereas all other tasks examined the rate of recovery for oiled and unoiled substrates which were initially organism free, in this task experiments were conducted on substrates which were allowed to colonize for a period of nine months prior to application of treatments. The specific objectives were to evaluate treatment effects from: oil (two levels); grazers (two levels); and tidal height (two levels). The effects were evaluated immediately after treatment, and five days and 30 days after field placement. Three independent experiments were conducted to evaluate the three intervals. The end points were tests of hypotheses for treatment differences in dry weight plant biomass, major group densities and species richness. An additional objective was to compare the partitioning of petroleum hydrocarbon concentration between the substrate itself and contained organisms.

The final task, Task I, had the principal objective of evaluating oil treatment effects on reseeding by larval littleneck clams (Protothaca staminea). A single experiment, of three months duration, during the late spring and summer had exactly the same configuration and objective end points as did Task B, and ten primary species were evaluated.

SECTION 2

CONCLUSIONS

GENERAL

1. Experimental studies of the effects of Prudhoe Bay crude oil on the recovery of infauna and epifauna of the intertidal zone of the Strait of Juan de Fuca were conducted over a two-year period. The studies involved field placement of oil-treated and untreated substrates (trays of sediment and concrete bricks) at four sites, three tide levels, and in two recruitment seasons. For infaunal experiments, the numbers and kinds of animals colonizing sediments after 15 months of field exposure closely resembled the numbers and kinds of animals reported from similar habitat at adjacent baseline sampling stations. The similarity extended beyond overall numbers and kinds of animals since the relative distribution of numbers and kinds within the major taxa also closely paralleled that reported for baseline stations. From those data it is concluded that the 15-month control sediments were fully recovered and that they reasonably represent what one would find by sampling uncontaminated sites on the Strait of Juan de Fuca with similar habitat. For the commercial clam bed, the molluscan herbivores and suspension-feeders were at least as sensitive to oil as detritivores. In the rock habitat, detritivores were not sensitive to the effect of oil treatment but herbivores and suspension-feeders were highly sensitive.

2. Effects from the oiling on recovery is strongly related to feeding type but the influence is different depending on habitat. For the sand habitats, detritivorous and herbivorous species were almost universally influenced by the oiling. Carnivorous species were about evenly divided in their response to the oiling and, with one exception, no significant effect was seen on the recovery of a suspension feeder.

3. Based on adjunct MESA studies of trophic relationships, it appears that the severity of influence on recovery of species in this study could be expected to have a deleterious effect on important fish populations, and that this effect would extend somewhat beyond the 15-month period studied here.

4. Because oil was mixed into sediment for infaunal studies, the present case may be considered a "worst" case situation in terms of treatment severity. However, the sediment-borne concentrations of both total oil and analyzed aromatic and saturate compounds were well within the range of concentration reported for sediments exposed to actual spillages elsewhere. Initial target concentrations of 2000 ppm for summer-fall, 3-month recovery and 15-month recovery and 1000 ppm for spring-summer recovery were obtained. Reductions in total hydrocarbons were about 35% in three months for summer-fall regardless of sediment type. The comparable amount for

MLLW in the spring-summer period was 43%. There was a slightly more rapid loss at the lower tide level (53%). At 15 months, total hydrocarbons were reduced by 85 and 97% for coarse and fine sediments, respectively. Based on rate of loss data between 3 months and 15 months, it is speculated that background levels would be reached in a total of 18.5 months. An important contribution to sediment infrared spectra (most likely due to biogenic materials and unrelated to oiling) was identified in analyses of control sediments after 15 months.

ESTIMATED RECOVERY TIMES

1. For the sand habitats investigated at Sequim Bay and Protection Island, a full recovery of infauna from the effects of an initial experimental oiling of 1,758 ppm total Prudhoe Bay crude oil was estimated to be 31 months.

2. Using the 15-month recovery of untreated sediments as a definition of full recovery, 3-month recoveries in similar sediment type, site and tide conditions were 69% for summer and 82% for fall in terms of numbers of species, and only 11% for summer and 18% for fall in terms of numbers of individuals. In 15 months, oil-treated sediments had recovered more than 90% in terms of numbers of species but had recovered only 48% in terms of numbers of individuals.

3. Although effects from oiling on recovery were found at each of the tide levels (MLLW and -2'[0.61 m]), in sand habitats at each of the sites (Protection Island and Sequim Bay), in each of the sediment types (Sequim Bay native and Protection Island native), and in each of the seasons (spring-summer and summer-fall), these physical factors, nevertheless, influenced gross density in both treated and control sediments. Thus, much higher densities were found in the summer-fall season than in the spring-summer season; much higher densities were found at Sequim Bay than Protection Island; and much higher densities were found at -2' below MLLW as compared to MLLW tide level. Density related to sediment type tended to be species specific and was about equal overall. Studies designed to elicit effects on recovery in an actual oil spill event will, thus, need some form of experimental control for these variables.

4. For the commercial clam bed habitat, a full recovery of the general infauna community from the effects of an initial experimental oiling of 2,500 ppm total Prudhoe Bay crude oil, was estimated to be 46 months. Recovery of the primary species, the littleneck clam (Protothaca staminea) may be slower because of depth stratification of oil as detailed below.

5. For the experimental concrete habitats, a full biological recovery cannot be predicted because of the lack of a usable definition of full recovery. Estimated "best" and "worst" cases for reaching background concentrations of total Prudhoe Bay crude oil at an initial treatment concentration of 8.72 grams per substrate unit (approximately 500 grams per square meter) were three and 13 months, respectively.

6. Predicted differences in recovery time for infauna between the sand and commercial clam bed habitats related strongly to initial concentrations of Prudhoe Bay crude oil and tide level.

7. Predicted recovery time for epifauna on concrete substrates did not bear a direct relationship to the amount of oil applied to experimental substrates.

RETENTION OF OIL IN THE EXPERIMENTAL SUBSTRATES

1. A ranking of substrates in retention of oil from higher to lower is (1) the commercial clam bed habitat; (2) sand from Sequim Bay and Protection Island habitats; and (3) concrete bricks used to represent rock habitat.

2. Experimental substrates of all kinds which were placed higher in the intertidal zone retained more oil than those of similar kind placed lower in the intertidal zone.

3. For the commercial clam bed substrate, more oil was retained at greater substrate depth.

4. For the sand substrate, a high proportion of total initial concentration of oil (about 50%) was lost from experimental substrates in three months time. Analyzed saturate compounds were lost from substrates at about the same rate as total oil. Analyzed aromatic compounds were lost much more quickly.

5. For the commercial clam bed, a much lower proportion of initial oil concentration (about 13%) was lost from experimental substrates in three months time. Analyzed saturate compounds were lost from substrates at about the same rate as total oil. Analyzed aromatic compound concentrations had not changed from initial concentrations in three months.

6. For a combination of the sand and sandy mud substrates, the retention of oil was closely tied to the initial concentration applied and tidal height of field exposure.

7. For the rock habitat represented by concrete bricks, a high proportion (84%) of total oil on bricks was lost in five days.

8. For each of the types of substrate, the total oil concentration, and concentrations of analyzed saturate and aromatic compounds, were well below concentrations reported in actual oil spill events.

SIGNIFICANT BIOLOGICAL EFFECTS FROM THE OIL TREATMENTS

1. The mean magnitude of 70% of 56 biological parameters estimated in these experiments was significantly reduced by the oil treatments.

2. Two species, because of their nearly ubiquitous occurrence within the recovery experiment and the north Puget Sound region, generally have been identified as good recovery indicators when used in an appropriate experimental framework. These species are the crustacean, Leptochelia dubia, and the polychaete, Exogone lourei. A taxonomic problem with the former and an important oil-resistant congener of the latter were identified. The species were verified to be useful in the commercial clam bed habitat. Exogone lourei should not be used at tidal heights above MLLW.

3. In the commercial clam bed habitat, and in the rock habitat, mollusks were especially abundant, and sensitive to oil treatment. The magnitude of effects from oil on mollusks often exceeded the magnitude of effects due to tidal height in these habitats.

4. In the commercial clam bed habitat, the density of the littleneck clam, Protothaca staminea, was significantly reduced by oil treatment.

METHODOLOGICAL VALIDATION

1. To protect the validity of statistical procedures, 13 species were selected as "primary" to evaluate effects on recovery in terms of individual species density. The numbers of individuals of the primary species comprised as very substantial proportion of all individuals in this study (78%) as well as at the Beckett Point baseline station (73%). They represented 33% of all individuals at the Jamestown baseline station for comparable conditions. Three-month control recovery for these primary species in terms of numbers of individuals closely paralleled that seen for all individuals (19% fall; 8% summer). The primary species represent the three major taxonomic categories quite well (polychaetes, crustaceans, mollusks).

2. The native and artificial substrates used in these studies proved to be a highly satisfactory means for discriminating between natural and pollutant effects in an experimental framework.

SECTION 3

RECOMMENDATIONS

The data in this study verify the utility of experimental approaches to measuring recovery of intertidal fauna following insult by sediment-borne pollutants. There are some specific studies using the approaches which have high priority and are recommended below based on the present findings.

In the realm of oil pollution research, the following should be investigated:

1. Supplemental studies should be conducted to characterize the organic and inorganic constituency of substrates used in these studies. Appropriately preserved samples are available.

2. Further sampling of substrates in place on the commercial clam bed evaluated in these studies is warranted. Both chemical and biological evaluation should continue for a period of no less than three months. Based on findings from those samplings, subsequent sampling may be indicated.

3. A less severe treatment with Prudhoe Bay crude oil should be used to bracket potential effects on recovery. The reduction in severity should relate to the method of applying oil and not necessarily the total amount used which, in the present case, was slight. Thus, an approach where oil is layered onto the surface of sediments, either from a seawater surface slick or direct surface application would be appropriate.

4. Comparative studies of the effects of recovery from processed petroleum products, i.e., light fuel oils and residual fuels should be undertaken in areas of the north Puget Sound region especially vulnerable to such spillage.

5. The relative severity on recovery effects following oil and oil dispersant combinations should be investigated using the present approach to assist decision making regarding the application of dispersants should spillage occur in our region.

The methods used in this study appear particularly suitable for investigation of other sediment-related pollutant problems in the Puget Sound region. Specifically, we recommend studies of the effects on recovery from:

1. Heavy metal contamination
2. Synthetic organic contamination
3. Dredge spoil contamination
4. Wood fiber and by-product contamination
5. Combinations of the above

Since the methodological sensitivities are a function of the prevailing seed populations and the types of physical factors identified in this study, there is good reason to believe that the approach used here would be appropriate for these studies.

The present studies have clearly identified an urgent need for two further types of investigation to assist in the interpretation of the effects demonstrated:

1. Basic life history studies of especially oil-sensitive primary species to include studies of behavior in response to pollutant contamination. Two questions need to be addressed: (1) what is the zonal distribution of the seed source; and, (2) where do organisms go that are absent from oil-treated sediments?

2. Experimental studies of feeding relationships, particularly of bottom-feeding flatfishes. The experiments should have a field orientation.

SECTION 4

MATERIALS AND METHODS

STUDY SITES

The experimental recovery studies used the four sites identified on Figure 1. The principal site is near the mouth of Sequim Bay, Washington. The natural substrate at this site is coarse sand, sparsely interspersed with cobble. The beach is east facing, well protected from northwesterly winds and ocean swell, and somewhat protected from the prevailing southeasterly winds by Travis Spit (Figure 1). Historically, the beach had served as a commercial source for both littleneck (*Protothaca staminea*) and butter (*Saxidomus giganteus*) clams. For the past 12 years, the beach has been owned by Battelle-Northwest and harvest of clams has been largely prohibited. It is now characterized by moderate populations of relatively large individual clams (Vanderhorst and Wilkinson, 1979). Parts of Tasks A through E and Tasks G and I were conducted at this site. The site served as a source for sand substrate identified as "coarse" sand which was evaluated *in situ* and also transposed in Task B to a second site on Protection Island (Figure 1). The Protection Island site is on a south-facing beach of fine sand. The beach is well protected from northwesterly winds and ocean swell, but was highly exposed to southerly winds. The fine sand beach was mobile during winter months and was used only for Task B during a late summer-early fall recruitment period. This site served as a source for "fine" sand, evaluated *in situ* and also transposed to the Sequim Bay site for use in Tasks B and C.

A north facing beach at Rocky Point (Figure 1) served as a third experimental site. The natural substrate at this site consisted mainly of cobble, with a mature, highly diverse, exposed rocky shore community. The beach is exposed to northwesterly storm winds. It was chosen as a comparative site for Task G during peak spring and summer recruitment periods to allow evaluation of site effects on availability of rocky shore larval forms at Sequim Bay. Finally, a fourth experimental site was on an actively managed commercial clam bed (Figure 1). The beach at Carr Point is southeast facing and subject to exposure from southerly winds. The severity of exposure to these winds is less than for the Protection Island site because of much reduced fetch. Substrate at this beach is a mix of fine and coarse sand and gravel.

EXPERIMENTAL APPROACHES

An experimental approach was adopted to investigate the effects of Prudhoe Bay crude oil on recovery of intertidal fauna principally because controlled experimentation seems to us the only practical way to discriminate between effects on faunal recovery due to oil and effects from other factors. The use of experiments in which controlled treatments are applied to the substrate itself permits a chemical characterization of the treatment. In the experiments reported here the substrates have been arrayed in such a manner to evaluate the effects from certain other environmental variables (e.g., tide level, site, season, substrate type) on a specific aspect of recovery. In studies of "natural" assemblages these factors are invariably confounded with effects of a contaminant of interest because true replication is unattainable in nature. The specific experimental approaches used in these studies also circumvent the problem of dependencies in time series observations of the same biological material. These dependencies are inherent in any study of "natural" populations and violate the most basic assumptions of statistical analysis if estimates of true error probabilities are needed. In the present studies, time series observations are circumvented by having a high replication of "blank" or organism-free substrates within each treatment category, and having the field exposure time fixed and equal for all treatment categories within an experiment. Our approach makes the assumption that the source organisms are equally available to all substrates (treated and untreated) and uses the high replication in untreated substrates to test that assumption. Thus, differences in the mean kinds and densities of organisms in or on substrates at the conclusion of an experiment should relate only to the treatments applied.

The experimental approach used is not without shortcomings. Since it is based on the assumption that the source and condition of organisms available to colonize substrates are unaffected by the treatment (a function of the treated substrate and not surrounding substrate or the water mass), the approach does not address perhaps important questions of recovery associated with that availability. The experimental approach used in these studies does not allow us to discriminate whether the source of organisms which colonize substrates is the water mass or surrounding substrate. There is some recent evidence (Santos and Simon, 1980) which suggests that trays placed on the bottom are colonized by larger, perhaps adult, organisms, as compared to trays of sediment suspended in the water column. These sorts of questions do not interfere with the validity of the experimental approach we have used but they do have a bearing on the overall question of rate of recovery following an actual oil spillage.

The approach in Tasks A through E and Task I used trays of sediment as experimental units. For Tasks G and H, concrete construction bricks were used for a similar purpose. In all cases (with the exception of Task H) the units were: (1) initially free of organisms; (2) treated with oil (treated) or not (control); (3) returned to the intertidal zone; and (4) allowed to colonize in a selected array of natural intertidal habitat conditions. The power of this approach lies in: (1) an equal starting point (organism free) for treated and untreated substrates; (2) the use of strict random procedures (as opposed to haphazard) for allocation of substrate units within and between treatments; (3) the inclusion of important organism-controlling variables (site, tide level, substrate type, season) as treatment categories (receiving both oil-treated and untreated units); and (4) the use of a replication scheme in the case of Tasks A through E and I for which methodological sensitivity had been preevaluated (Vanderhorst et al., 1978).

Balanced experimental designs were used in all the experiments reported here, and independent controls were used in each phase of each experiment; thus, given the inherent assumptions of normality, common variance, and additivity of the statistical model, a correct use of analysis of variance is indicated. This is in sharp contrast to the use of analysis of variance in field surveys for descriptive purposes in which time series data create dependencies between treatment categories and alter error probabilities in an undefinable manner.

INFAUNAL STUDIES

Details of the experimental methods including criteria for site selection, preparation and placement of substrates, chemical and biological characterization, and sampling rationale and procedures have been previously reported for the infaunal studies (Vanderhorst et al., 1979; 1980). In summary, native substrate was collected from three sites (Figure 1), brought to the laboratory, and given a repeated freezing-thawing treatment to kill macrofauna. Half of the substrate from each site was treated with Prudhoe Bay crude oil by mixing in a commercial cement mixer. A target concentration of 2,000 ppm total oil was sought in Tasks A, B, C, and I, and a target concentration of 1,000 ppm total oil was sought in Tasks D and E. Total amounts of oil and concentrations of selected petroleum compounds were measured in treated and untreated sediments prior to field installation, at intervals between installation and completion, and upon completion of a given experiment. The other half of the substrate from each site served as control. It received the mixing just as the oiled substrate but did not receive an application of oil. The prepared substrates were placed in PVC trays (30 x 15 x 15 cm). The bottom of the trays were provided with eight 2.5 cm diameter holes for drainage. Experimental substrates were retained in trays by placing a fiberglass screen over these holes. For Tasks A, B, C, and E, trays were buried with top surface flush with the ground surface at MLLW at each of the sites. For Task D, trays were buried in a similar fashion at -2' below MLLW at the Sequim Bay site. For Task I burial of trays was at MLLW and +2' above MLLW. Field installations for Tasks A, B, and C were during August 1978. Field installations for Tasks D and E were during April 1979. Task B terminated in November 1978. Tasks D and E terminated in August 1979. Task I was initiated during May 1980 and terminated late July 1980. Tasks A and C terminated in November 1979.

A relatively high amount of replication was used in both the placement and sampling of substrate units (Tables 1 and 2 list sampling dates). Based on a predesign study using similar units (Vanderhorst et al., 1978), this replication was done to permit evaluation of methodological sensitivity and to attain a quantitative measure of the density of individual species colonizing the trays. Because of interest in a large number of species and a limited number of independent units in even this rather large

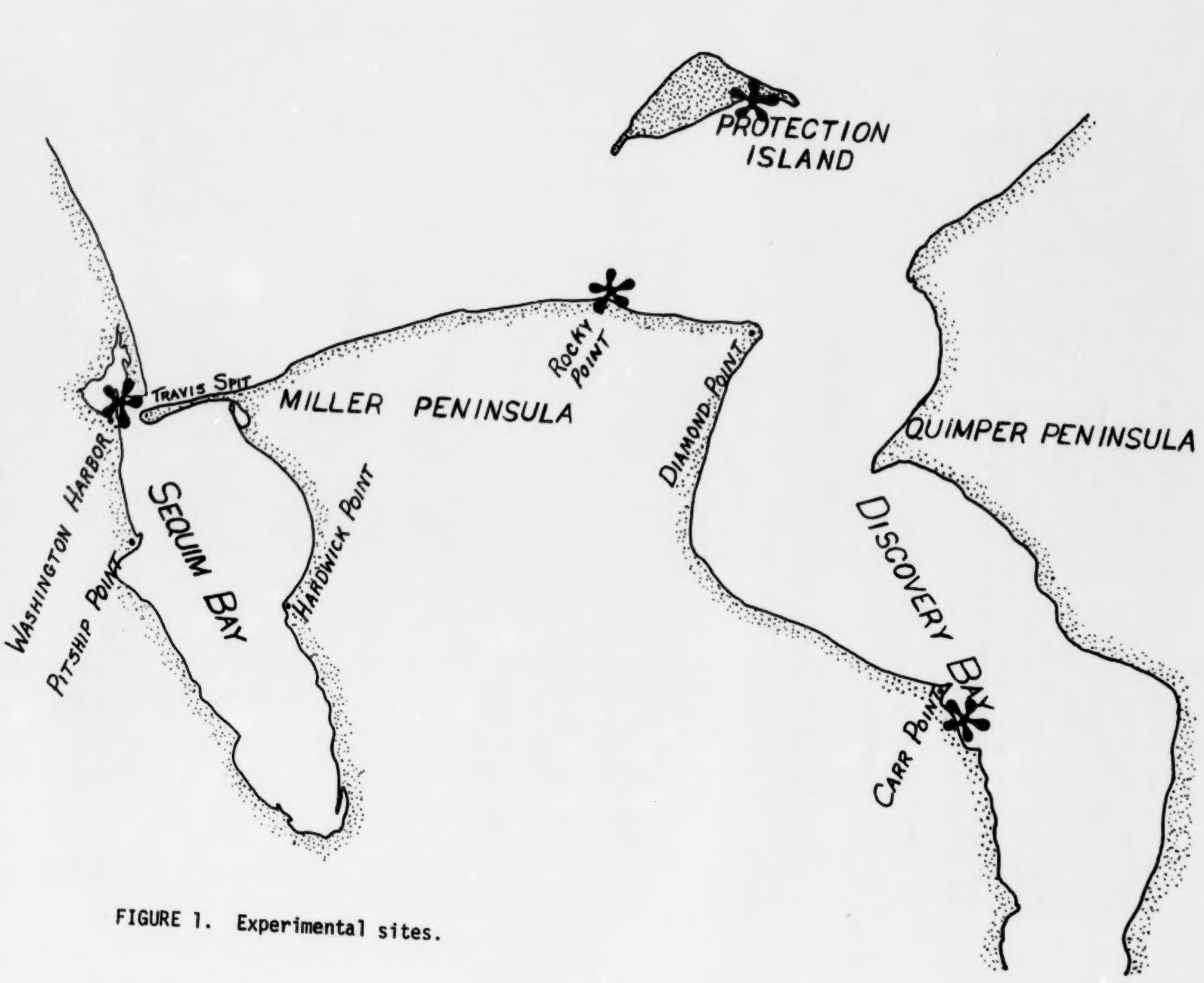
PROTECTION ISLAND
QUIMPER PENINSULA
ROCKY POINT
DIAMOND POINT
TRAVIS SPIT
MILLER PENINSULA
DISCOVERY BAY
CARR POINT
WASHINGTON HARBOR
SEQUIM BAY
HARDWICK POINT
PITSHIP POINT

FIGURE 1. Experimental sites.

design, it was necessary to *a priori* select species of special interest for hypothesis testing with valid probability statements concerning statistical error. For these species we use a criterion of $\alpha \sim 0.01$ to deem "significant" effect on density. The maximum real probability for Type I error for any one of the seven species (Task B) was 7%, and for any one of the ten species (C, D, E, I) was 10%. Task A data were outside the experimental framework. To meet the objectives of Tasks B, C, D, E, and I, four independent experiments were conducted and *a priori* selection of target species was made. These species were designated "Primary" species and consist of the following:

For Experiment I

(Task B):
- Mollusks
  - *Mysella tumida*
  - *Transennella tantilla*
  - *Lacuna* sp.
- Polychaetes
  - *Platynereis bicanaliculata*
  - *Armandia brevis*
  - *Ophiodromus pugettensis*
  - *Capitella capitata*

For Experiment II
(Tasks D and E) and

For Experiment III
(Task C): and

For Experiment IV
(Task I):
- Mollusks
  - *Mysella tumida*
  - *Protothaca staminea*
  - *Lacuna variegata*
- Polychaetes
  - *Platynereis bicanaliculata*
  - *Armandia brevis*
  - *Polydora socialis*
  - *Exogone lourei*
- Crustaceans
  - *Leptochelia dubia*
  - *Corophium ascherusicum*
  - *Photis brevipes*

The basis for *a priori* species selection for Task B rested on: (1) results from the predesign study (Vanderhorst et al., 1978); and (2) for two species, *Lacuna* sp. and *Capitella capitata*, reported perturbations following oil spills elsewhere. The basis for *a priori* selection of

Table 1. Schedule of sampling for oil recovery experiments.

MONTH/YEAR	SITE/STATUS TIDE LEVEL ( )	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
August/1978	Preliminary	ABC	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
				Fine	Infrared	3	9
					Gas chromat.	3	3
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
				Fine	Infrared	3	9
					Gas chromat.	3	3
September/1978	Protection Is. (0')	A	Oiled	Fine	Infrared	1	1
					Biological	1	1
			Unoiled	Fine	Infrared	1	1
					Biological	1	1
	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
October/1978	Protection Is. (0')	A	Oiled	Fine	Infrared	1	1
					Biological	1	1
			Unoiled	Fine	Infrared	1	1
					Biological	1	1
	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
November/1978	Protection Is. (0')	B	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35

Table 1. (Continued)

MONTH/YEAR	SITE/STATUS TIDE LEVEL ( )	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
November/1978	Protection Is. (0')	B	Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
November/1978	Sequim Bay (0')	B	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
December/1978	Protection Is. (0')	A	Oiled	Fine	Infrared	1	1
					Biological	1	1
			Unoiled	Fine	Infrared	1	1
					Biological	1	1
	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
January/1979	Protection Is. (0')	A	Oiled	Fine	Infrared	1	1
					Biological	1	1
			Unoiled	Fine	Infrared	1	1
					Biological	1	1
	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1

Table 1. (Continued)

MONTH/YEAR	SITE/STATUS TIDE LEVEL ( )	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
April/1979	Sequim Bay (0')	A	Oiled	Coarse	Infrared Biological	1 1	1 1
April/1979	Preliminary	D,E	Oiled	Coarse	Infrared Gas chromat.	3 3	9 3
June/1979	Sequim Bay (0')	A	Oiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (0')	D(A)	Oiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (0')	A	Unoiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (0')	D(A)	Unoiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (-2')	E(A)	Oiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (-2')	E(A)	Unoiled	Coarse	Infrared Biological	1 1	1 1
July/1979	Sequim Bay (0')	A	Oiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (0')	D(A)	Oiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (0')	A	Unoiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (0')	D(A)	Unoiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (-2')	D(A)	Oiled	Coarse	Infrared Biological	1 1	1 1
	Sequim Bay (-2')	E(A)	Unoiled	Coarse	Infrared Biological	1 1	1 1

Table 1. (Continued)

MONTH/YEAR	SITE/STATUS TIDE LEVEL ( )	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
August/1979	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
August/1979	Sequim Bay (0')	D	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
August/1979	Sequim Bay (-2')	E	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
September/1979	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
October/1979	Sequim Bay (0')	A	Oiled	Coarse	Infrared	1	1
					Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
November/1979	Sequim Bay (0')	C	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
						254	788

species in Tasks C, D, E and I were: (1) results of Task B studies; and (2) examination of baseline data for nearby stations (Nyblade, 1979). The danger in a priori selection in any experiment involving field colonization is that selected species may not occur in the future experiment or may be relatively unimportant constituents. In general, this was not the case for the species selected here.

In addition to the 13 species which were a priori selected to protect the validity of error probability statements, quantitative data were also collected on the density of more than 200 other species which colonized trays. Analyses of variance were computed for these data for descriptive purposes.

Sediment Extraction and Chemistry

Sediment cores collected for chemical analysis were frozen immediately after collection. The frozen samples were thawed at room temperature and thoroughly mixed for subsampling. Twenty grams (wet) of sediment were placed in a 250 ml teflon-capped bottle with 20 g of anhydrous sodium sulfate and thoroughly mixed to hydrate the water from the sediment. To the samples for I.R. analysis, 50 ml carbon tetrachloride was added, and for capillary G.C. analysis, 25 ml hexane was added. The bottles were shaken overnight on a reciprocal shaker. The solvents were decanted from the sediment. Carbon tetrachloride was extracted into a 25 ml scintillation vial, and hexane was extracted into a 50 ml graduated cylinder. The CCl_4 subsample was analyzed by infrared spectroscopy (Simard et al., 1952). The samples for G.C. analysis were extracted for an additional two hours with 25 ml hexane and decanted into the graduated cylinder with the first extraction. The sediment sample was then extracted with 5 ml volumes of hexane and decanted until the total extract volume was 50 ml. Twenty-five ml of the hexane extract were concentrated under nitrogen to three ml and separated into aliphatic and aromatic fractions by silica gel chromatography (Warner, 1976). These fractions were concentrated to one ml and analyzed for individual hydrocarbons by capillary gas chromatography. Concentrations were calculated by using standard addition and internal standard compounds with comparison to authentic standards. Concentrations of individual aliphatic and individual aromatic compounds were summed to represent the respective groups.

Sediment Grain Size Analysis

Following chemical analyses of preliminary samples, the remainder of core samples for these analyses were retained in a frozen condition. Twelve replicate cores from Sequim Bay, 10 replicate cores from Protection Island, and 11 replicate cores from Discovery Bay were analyzed for sediment grain size. Frozen cores were thawed at room temperature and dried in an oven at 100°C for 48 hours. Following drying, individual cores were sorted to classes of particle size as follows. A series of standard sieves, mesh sizes: 5.66 mm, 2.0 mm, 1.0 mm, 500 μm, 125 μm, and 63 μm, were stacked on an Eberbach shaker. Sediment from a single core was emptied into the top sieve. The shaker was activated for a period of 10 minutes. The sediment retained on each sieve and in a pan placed below the finest sieve, was weighed on an analytical balance. The weight of sediment in each of the seven size classes for each individual core was then computed as a percentage of the total weight of sediment in that core.

EPIFAUNAL RECOVERY STUDIES

Concrete construction bricks (19.5 x 5.5 x 9 cm) were used as experimental substrates for studies of epifaunal recovery. The bricks have been shown to be suitable for colonization by a variety of typical rock epifauna in previous studies (Vanderhorst et al., 1975; Vanderhorst and Wilkinson, 1977). They have two additional advantages for the present studies in which more than 2,000 individual substrate units were evaluated. The bricks were readily available in large quantities with good uniformity in size, shape, and porosity. Also, they were amenable to placement on the beach without the need for a physical support system to keep them in place.

The experimental method involved four steps: (1) preconditioning of concrete construction bricks by placement in a laboratory flowing-seawater system for two weeks; (2) treatment of one-half of conditioned bricks with Prudhoe Bay crude oil; the other half served as controls; (3) characterization of treatment severity by extraction of oil from bricks and chemical analyses; and (4) field evaluation of oil content and biological colonization in month-long experiments. Independent experiments were conducted each month commencing with October 1979 through July 1980. Additionally, two identical experiments during June and July 1980 were conducted at Rocky Point to test the effect at that site.

Preconditioning of bricks in laboratory flowing sea water was to leach out any foreign materials which might have been associated with the manufacture of the bricks and to allow some chance for colonization with a microflora. Prior to the treatment phase, all bricks, both control and treated, received a thorough washing with a high-pressure hose both to remove unseen but possible incidental occurrences of settled macroorganisms and to aid in equalizing the effect of preconditioning on control versus treated bricks. For treated bricks, treatment lasted five days. During this time the control bricks remained in the preconditioning tank. At the end of the treatment phase, the control bricks again received a wash with the high-pressure hose.

The treatment of bricks with oil was designed to simulate repeated exposure of intertidal rock during shifts of the tide. The procedure was as follows. Conditioned bricks were placed on the bottom of a rectangular tank (0.54 x 4.88 m). The tank was then filled with sea water (25 cm depth). Prudhoe Bay crude oil (20 ℓ) was poured on the surface of the sea water to provide a slick thickness of about 1 cm. Continuous inflow of clean sea water was provided (two ℓ/min). Both the inflow and outflow of the sea water were subsurface to prevent disturbing the surface slick and to retain it in the tank. Twice each day the inflow of sea water was discontinued and the seawater level reduced to a depth of 2 cm. The period of this reduced water level was for two hours at each treatment. During the periods of low water, the slick was in contact with the upper and side surfaces of the bricks. The twice-daily regime was repeated for five days. At the end of five days, a surface outflow was provided, and the slick was washed into the laboratory oil-treatment facility. Clean seawater flow (two ℓ/min) was provided for a further 24 hours. Bricks were then placed in the intertidal zone for one month.

Chemical Characterization of Bricks

Routine chemical characterization of the treatment severity has been based on five brick subsamples (see Table 2 for schedule). Analysis methods for both infrared spectrophotometry and capillary gas chromatography follow those previously reported (Vanderhorst et al., 1979). Three types of extraction procedures have been evaluated and two are routinely used. The first procedure involved washing whole wet bricks with 500 ml CCl_4. The extraction efficiency was poor. The second procedure involved air drying bricks for a period of 48 hours before extraction with 500 ml CCl_4. This improved the extraction efficiency approximately 100%. To provide a better measure of the amount of oil actually "seen" by colonizing organisms, a two-part extraction procedure was adopted. In this procedure the top surface of bricks was washed with 200 ml CCl_4. The amount of oil in this extract was measured. The bricks were then air-dried for 48 hours and re-extracted with 500 ml CCl_4. The amount of oil in this extract was measured. Diluted samples of the extracts measured by infrared spectrophotometry are computed in terms of numbers of grams of oil per brick. Samples analyzed by capillary gas chromatography are reported in terms of number of milligrams per brick for individual compounds.

Biological Characterization of Bricks

For days one through five following placement of bricks at the appropriate tide level in the intertidal zone, observations of all bricks during extreme low tides were made to determine the presence or absence and, insofar as possible, kinds of organisms colonizing bricks. Thirty days after field placement all organisms were scraped from the top of 15 bricks in each treatment category (total 60 bricks). Animal species were identified and counted. A subsample of five bricks was used for chemical analyses described above.

GRAZER MANIPULATION STUDIES

Three experiments were conducted to examine effects from Prudhoe Bay crude oil, grazer manipulation, and tide level on epifauna and flora. The bricks used for these studies were randomly selected from a large pool of bricks colonized at MLLW and +2' above MLLW for nine months preceding the experiments (September 1979 through May 1980). The bricks were subdivided into eight treatment categories as follows:

Table 2. Preparation of Units and Sampling Schedule for Experiments on Effects of oil on Recovery of Commercial Clams and Epifauna on Rocky Intertidal.[1]

TASK/SITE	DATES	UNIT TYPE	PRELIMINARY	MLLW TIDE	+2 MLLW TIDE	TOTALS
A/Discovery Bay	5/80	Infrared				
		Trays	6			6
		Cores	18			18
		Capillary GC				
		Trays	6			6
		Cores	6			6
	6/80	Infrared				
		Trays		2	2	4
		Cores		2	2	4
		Biological				
		Trays		2	2	4
		Cores		2	2	4
	7/80	Infrared				
		Trays		2	2	4
		Cores		2	2	4
		Biological				
		Trays		2	2	4
	8/80	Infrared				
		Trays		6	6	12
		Cores		18	18	36
		Capillary GC				
		Trays		6	6	12
		Cores		6	6	12
		Biological				
		Trays		10	10	20
		Cores		70	70	140

Note: Core profile on capillary GC and Biological Cores means that discrete samples were 2X indicated number for 8/80 sampling.

Table 2. (Continued)

TASK/SITE	DATES	UNIT TYPE	PRELIMINARY	MLLW TIDE	+2 MLLW TIDE	TOTALS
B/Sequim Bay	9/79	Concrete				
		Infrared	10	10	10	30
		Capillary GC	10	10	10	30
		Biological[2]		30	30	60
	10/79 - 8/80	The pattern for Task B was followed each month with exception of Capillary GC, giving totals as follows:				
B/Rocky Point	5/80	Concrete				
		Infrared	10	10	10	30
		Biological[2]		30	30	60
	6/80	Concrete				
		Infrared	10	10	10	30
		Biological[2]		30	30	60
C/Sequim Bay	4/80	Concrete				
		Infrared	10	10	10	30
		Capillary GC	4	4	4	12
		Biological[2]	120	60	60	240
	5/80	Concrete				
		Infrared		10	10	20
		Biological		30	30	60

---

[1] Experiment balanced, i.e., for every treated sample a control sample is also indicated.

[2] Each of the biological units with this notation (with the exception of preliminary) should be multiplied by 5 for 5 daily observations within the month. Appropriate total unit samples are: Capillary GC, 60; Infrared, 532; Biological, 3328.

OILED BRICKS

MEAN LOWER LOW WATER

(1) Limpets Stocked
(2) Limpets Removed

PLUS TWO FEET ABOVE MEAN LOWER LOW WATER

(3) Limpets Stocked
(4) Limpets Removed

UNOILED BRICKS

MEAN LOWER LOW WATER

(5) Limpets Stocked
(6) Limpets Removed

PLUS TWO FEET ABOVE MEAN LOWER LOW WATER

(7) Limpets Stocked
(8) Limpets Removed

Bricks receiving oil treatment were treated exactly as bricks in the monthly epifaunal experiments described above. The nonoiled bricks were placed on a water table and received a continuous flow of seawater during the five-day oil treatment period. The tide level treatment involved placing bricks in the intertidal zone at the indicated tide level immediately after completion of the oil treatment phase. The grazer treatment involved placing ten limpets (*Acmaea* spp.), with an approximate shell diameter of two cm, on each brick in the grazer stock category and removing all visible limpets from the grazer removal category. This process was repeated several times each day throughout the five-day treatment phase. Stocked limpets in the oil-treated portion of the experiment were all dead after completion of two treatment cycles. Stocked limpets on the unoiled bricks tended to wander off bricks and onto the sides of the water table. In no case did we observe limpets on the "limpets-removed" category of substrates. Grazer treatment ended simultaneously with oil treatment at the time of field placement.

A subsample of ten bricks from the oil-treated categories was evaluated for total oil content at the completion of the oil treatment phase. The plant and animal material was scraped from five of these bricks prior to oil extraction and analysis. The other five bricks were extracted and analyzed with organisms intact.

Three independent experiments were based on samples of five bricks for each treatment category (total 40 bricks) at: (1) immediately after treatment; (2) five days after field placement; (3) 30 days after field placement. Likewise, subsamples of bricks were taken at each of the intervals for analysis for total oil.

The biological characterization of bricks in the experiments was done by completely removing living material by scraping. Animals were identified and enumerated by species. Plants were evaluated in aggregate, by brick, in terms of dry weight biomass. Plant material was oven-dried until asymptotic weight was reached.

SECTION 5

RESULTS

RECOVERY ON HARD SUBSTRATES

A Perspective

The dominant species on rock substrates are long-lived as compared to the infauna of sand and mud. This fact has prompted Nyblade (1979) to assign relative recovery times measured in decades for rock habitat as compared to a few years for the infauna communities. Coupled with this relatively long-term biological recovery (possibly involving successional processes), rock, as compared to mud and sand, presents a relatively minor surface area for accumulation and retention of spilled petroleum. These two factors in concert tend to suggest that the primary effects of oil on recovery of these communities will be the degree to which the complex biological communities are broken down by the effect of oiling. A third characteristic of the communities of this habitat is that the long-lived, dominant species tend to have infrequent successful recruitment. Periods between natural successful recruitments may be from three to seven years. Because of this factor, even a short-term impairment of substrate suitability for settlement may have far-reaching effects on direction of future recovery. The 12 short-term experiments in Task G were designed to test the effect of oiling organism-free hard substrates on the suitability of those substrates for successful settlement and survival. Thus, the data provided here do not directly address the actual recovery times for communities on hard substrates. Rather, they establish effect of oiling on an important first step in the recovery process. The three short-term experiments in Task H allowed for contaminant-free colonization of substrates and subsequent measurement of effects from oiling on the complexity of existing communities. Although these experimental communities were quite simple as compared to mature rocky shore communities, the experimental approach nevertheless provides an opportunity to determine whether or not oil applied to these communities is selective in effect for community components and to make inferences concerning subsequent recovery.

Hard Substrate Recovery - Biological Data Presentation

Two groups of experiments conducted to examine the effects of oil treatment on recovery of epifauna on hard substrates are treated separately. The first group consisted of 10 experiments, one each month during the period of October 1979 through July 1980. These were all carried out with Sequim Bay field exposure and are designated "monthly" experiments. One of the primary objectives of these experiments was to evaluate differences in recovery related to season of field exposure. The other group consisted of two experiments, designated as "site" experiments. These were conducted at Rocky Point during the months of June and July,

1980. The methods for these two experiments exactly paralleled the two "monthly" experiments at Sequim Bay for these two months only, and comparisons between the two sites of field exposure are made in the section on "site" experiments.

Monthly Experiments. Each of these 10 experiments was independent of the others in the group, i.e., each was set up using preconditioned, but uncolonized bricks. Sixty bricks, 15 in each of four treatment categories (1 = oil-treated MLLW; 2 = oil-treated +2' above MLLW; 3 = untreated MLLW; 4 = untreated +2' above MLLW) were used for biological analyses in each of the 10 experiments. Differing total numbers of bricks (70 or 80 bricks) were used in individual experiments depending on the schedule for chemical analysis.

The first biological data collection in each of the 10 monthly experiments involved examination of each of the individual bricks once each day during periods of extreme low tide for the first five days of field exposure. The goal of these observations was to determine the earliest time at which marine larvae settled on bricks. The results from these 3,000 individual brick examinations (over the 10 experiments) consisted of a few random occurrences of mobile adult organisms. In no case was an organism which could be identified as an attached marine larva observed on any brick. A tabulation of calendar months in which the different kinds of organisms were observed is given below:

TAXA	CALENDAR MONTH 1979	 1980
POLYCHAETES		
Polychaetes undet.	10, 11	1, 2, 3, 4
CRUSTACEANS		
Amphipods undet.		2,
Exosphaeroma amplicauda		3, 4
Exosphaeroma sp.		1, 5, 6, 7
Hemigrapsus sp.		3, 5
Hermit crab		3
MOLLUSKS		
Lacuma sp.	12	1, 2, 6, 7
Limpet	9, 10	2, 3, 4, 5
Mopalia lignosa		1

The remainder of biological data for the 10 monthly experiments resulted from scraping all fauna from each of the 60 bricks in each experiment at the end of 30 days of field exposure (total = 600 bricks).

Since the monthly experiments were all independent, we deemed it most appropriate to use a single analysis of variance covering all 10 experiments for each response variable of interest. The response variables were evaluated in the experimental design model:

$$Y_{ijk} = M_i + O_j + T_k + E$$

where: Y = the response variable magnitude;

M = the main effect on response variable magnitude due to month of experiment (i = (1 = October 1979 through 10 = July 1980));

O = the main effect on response variable magnitude due to oil treatment (j = (1 = oil-treated; 2 = untreated));

T = the main effect on response variable magnitude due to tide level of field exposure (k = (1 = MLLW or 2 = +2' above MLLW)); and

E = random error.

The interactions between main effects on response variable magnitude are also of interest and have been computed. The tests for statistical significance concerning interaction means are, however, not included in this report because of interpretive difficulty concerning the interactions.

The response variables evaluated (Y's) in the model included numbers of individuals within taxonomic and trophic categories and numbers of specific entities within taxonomic categories. For convenience sake we refer to specific entities as "species" throughout this report. In fact many of the specific entities are undetermined species within a genus or higher taxa, and in a few cases, specific entities are fragments. Additionally, the number of specific entities per brick was estimated using the model. Hypotheses were tested for non-zero differences due to month, oil treatment, and tide level.

The composition, trophic classification, and an abbreviated analysis of variance for individual numbers of mollusks are shown in Table 3. There were 20 species of mollusks identified from bricks in the ten monthly experiments. Of these, 9 (45%), were herbivores, 7 (35%) were suspension-feeders, 3 (15%) were carnivores, and 1 (5%) was a parasite. The trophic classification for the species comes from Simenstad et al. (1979). The composition indicated on Table 3 includes many species which are normally associated with rock habitat including several species of grazing snails and limpets, and the suspension-feeding dominant mussel, _Mytilus_ _edulis_.

Table 3. Species composition and analysis of variance for mollusk density in monthly hard substrate recovery experiments at Sequim Bay.

COMPOSITION

SPECIES	TROPHIC CATEGORY[1]
Acmaea digitalis	herbivore
Acmaea pelta	herbivore
Acmaea persona	herbivore
Acmaea sp.	herbivore
Alvania compacta	herbivore
Alvania sp.	herbivore
Chlamys rubita	suspension
Clinocardium nutallii	suspension
Cooperella subdiaphana	suspension
Doto sp.	carnivore
Lacuna sp.	herbivore
Littorina scutulata	herbivore
Margarites sp.	herbivore
Mopalia muscosa	carnivore
Mysella tumida	suspension
Mytilus edulis	suspension
Nucella (Thais) sp.	carnivore
Odostomia sp.	other
Protothaca staminea	suspension
Transennella tantilla	suspension

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[1] Trophic classification after Simenstad et al. (1979).

ANALYSIS OF VARIANCE FOR
INDIVIDUAL NUMBERS PER BRICK

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[2]
Month	9	6.705	Yes
Tide Level	1	4.184	No
Oil Treatment	1	12.822	Yes
Error	550	1.242	

[2] Probability for Type I error is equal to or less than 0.01.

It is apparent that several of the species indicated in the composition are ones which are normally characteristic of soft substrates, particularly including several species of suspension-feeding clams. The abbreviated analysis of variance indicates significant ($p = 0.01$) effects on density of mollusks due to month and due to the oil treatment. Significant effects due to tide level were not demonstrated.

Figure 2 shows the mean densities of mollusks by month, oil treatment, and tide level. Peak densities of mollusks were in March, followed closely by June. Numbers of mollusks on bricks were lowest in January and February. The maximum mean difference in density of mollusks was between March and January, and far exceeds mean differences due to the other sources. The significant effect due to oiling (C-O, Figure 2) is greater than the difference shown for tide level (+2'-0).

The species composition, trophic groups, and an abbreviated analysis of variance for numbers of individual crustaceans are shown in Table 4. There was a greater number of crustacean species as compared to mollusks (Table 3). The trophic structure of the group also differed, with detritivores making up 35%; herbivores, 23%; suspension-feeders, 16%; carnivores, 16%; and the "other" category contributing 10%. Thus, the 31 species were more evenly distributed than mollusks in trophic composition. Although the barnacles were represented by two species (Table 4), the composition of crustaceans found associated with the bricks reflects mostly transitory species.

Because a high proportion of individual numbers of crustaceans per brick were represented in a single species, Exosphaeroma sp. (a scavenging, herbivorous isopod), the data on this species were removed from crustaceans as a group for the analysis of variance shown in Table 4. The analysis reflects significant effects on density of crustaceans due to month of experiment and tide level. Significant effects due to the oil treatment were not demonstrated.

The largest peak in numbers of individual crustaceans per brick was seen in October 1979 (Figure 3). A much smaller, but distinct, peak in numbers was seen in March 1980. Mid-winter and mid-summer numbers were quite low. The significant tide level effect on numbers of crustaceans indicates a much higher number at the MLLW level. This difference due to tide level is much greater than the difference due to oiling. It is interesting, although not statistically significant, that the apparent effect of the oiling was to reduce the number of individual crustaceans.

The analysis of variance for Exosphaeroma sp. alone follows exactly the same pattern as did that for remaining crustaceans. The data, in Table 5, indicate significant effects on density due to month of experiment and tide level, but do not demonstrate a statistically significant effect due to oil treatment.

The peak in density for Exosphaeroma sp. occurred in the March 1980 experiment (Figure 4). High numbers were observed also in October and

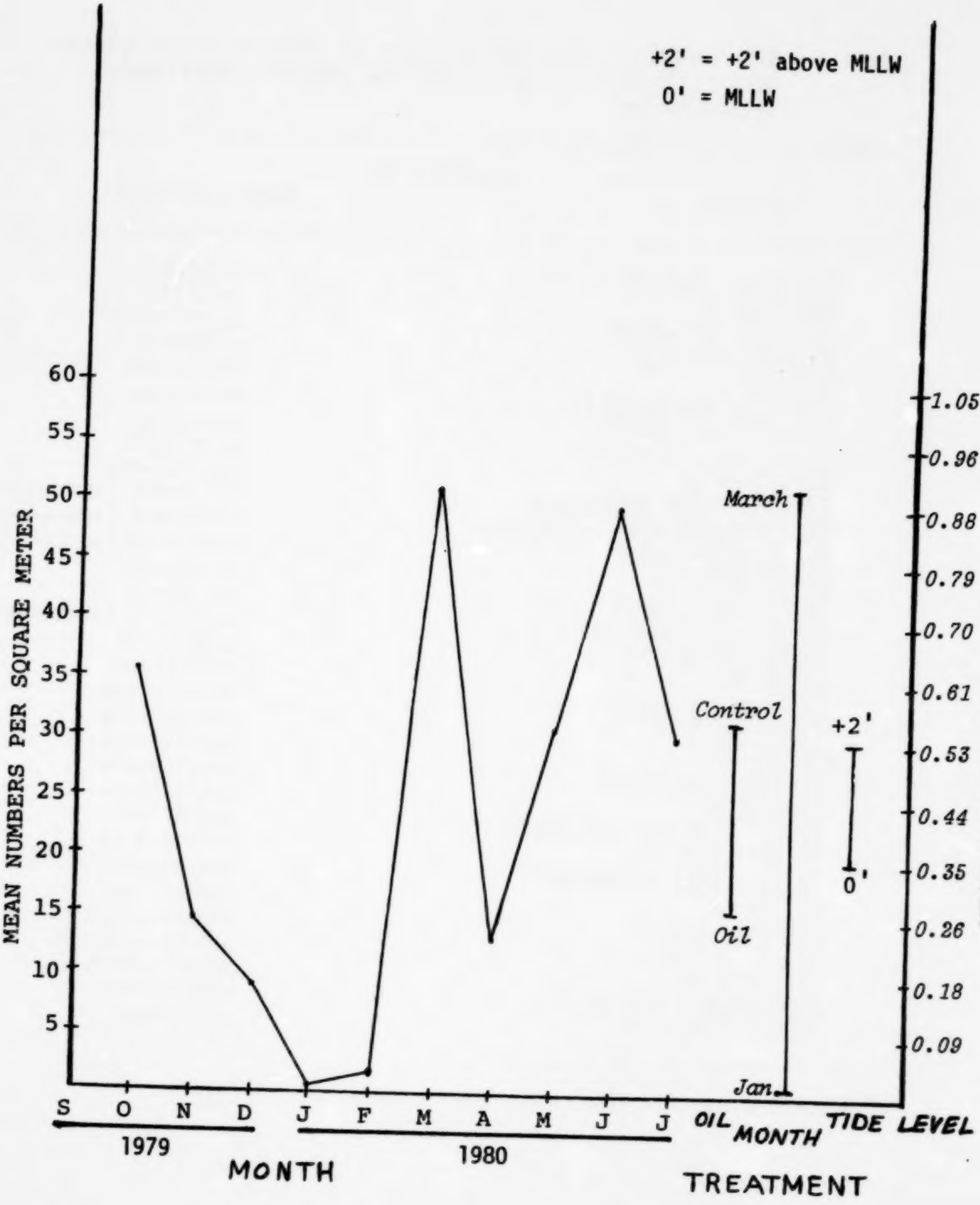

Figure 2. Mean densities for mollusks in numbers per square meter by month and in numbers per brick by treatment.

Table 4. Species composition and analysis of variance for crustacean density in monthly hard substrate recovery experiments at Sequim Bay.

SPECIES	COMPOSITION TROPHIC CATEGORY[1]
Ampithoe simulans	herbivore
Ampithoe sp.	herbivore
Aoroides columbiae	detritivore
Balanus crenatus	suspension
Balanus sp.	suspension
Caprella laeviuscula	herbivore
Caprellidae (undet.)	herbivore
Cancer sp. (larval)	carnivore
Corophium sp.	detritivore
Exosphareroma amplicauda	herbivore (scavenger)
Gnorimosphaeroma o. oregonensis	herbivore (scavenger)
Hemigrapsus nudus	carnivore (scavenger)
Hemigrapsus sp.	carnivore
Heptacarpus nudus	carnivore
Idothea wosnesenskii	herbivore (scavenger)
Ischyrocerus sp.	suspension
Jassa sp.	detritivore
Leptochelia dubia	detritivore
Leptochelia sp.	detritivore
Melita sp.	detritivore
Orchomene pacifica	detritivore
Pagurus sp.	detritivore
Parallorchestes ochotensis	detritivore
Petrolisthes sp.	detritivore
Petrolisthes eriomerus	suspension
Photis sp.	suspension
Pinnixia eburna	others-parasite
Pinnixia faba	others-parasite
Pinnixia sp.	others-parasite
Pontogeneia inermis	detritivore
Shrimp fragments	carnivore

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[1] Trophic classification after Simenstad et al. (1979).

Table 4. (Continued)

SOURCE	ANALYSIS OF VARIANCE FOR INDIVIDUALS PER BRICK		
	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[2]
Month	9	1724.0	Yes
Tide Level	1	3204.6	Yes
Oil Treatment	1	49.7	No
Error	580	42.4	

[2] Probability for Type I error is equal to or less than 0.01. Analysis of variance excludes _Exosphaeroma_ sp. which is treated separately.

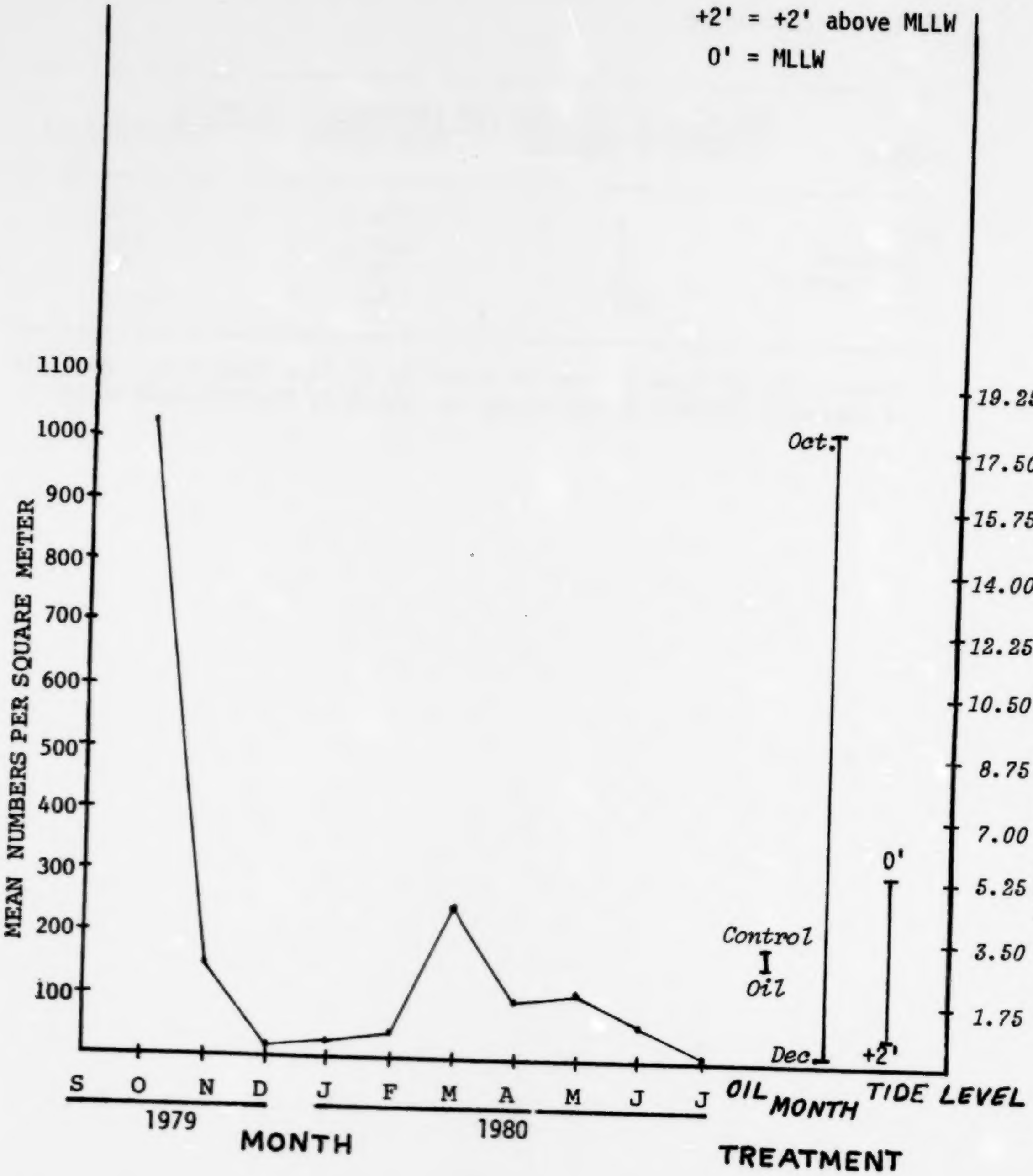

Figure 3. Mean densities for crustaceans excluding Exosphaeroma sp. in numbers per square meter by month and in numbers per brick by treatment.

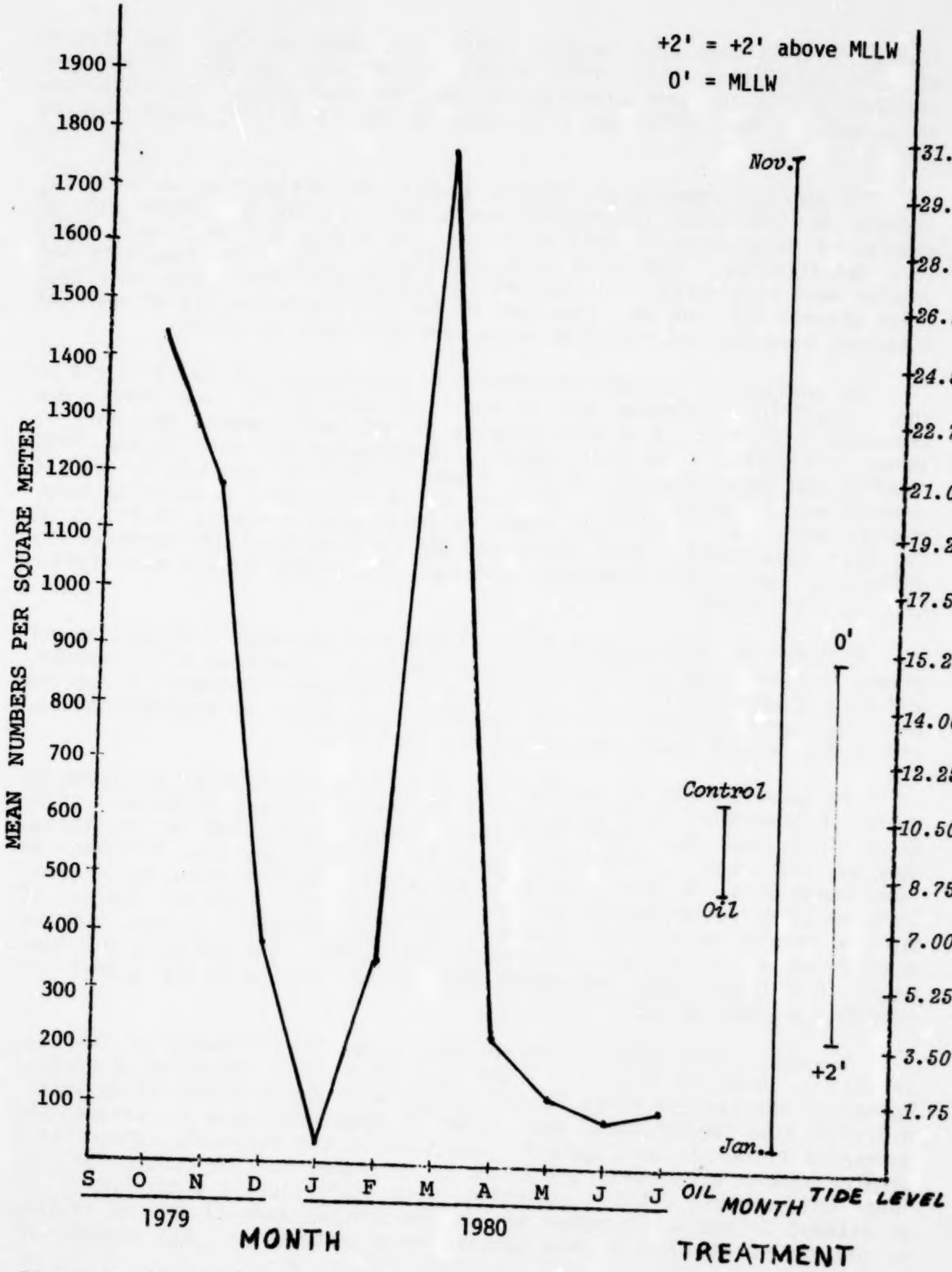

Figure 4. Mean densities for Exosphaeroma sp. in numbers per square meter by month and in numbers per brick by treatment.

November, 1979, and much reduced numbers in other months. The statistically significant tide level effect on density appears to be even stronger and in the same direction as the tide level effect on crustaceans as a whole. Mean differences in numbers due to oil treatment favored control bricks.

The species composition, trophic groups, and analysis of variance for numbers of individual polychaetes are shown in Table 6. There were 21 species of polychaetes overall on bricks. Of these, more than half (52%) were detritivores. One-third were carnivores (33%). The remaining two species were herbivores. The composition somewhat reflects, and individual brick observation confirms, that most of the polychaetes on bricks were not attached; even the tube-building forms were transient in nature.

The analysis of variance of numbers of polychaetes per brick (Table 6) shows significant effects due to month of experiment, tide level, and treatment with oil. A graphic display of the mean numbers per brick is shown in Figure 5. The peak number of polychaetes was seen in the first monthly experiment (October 1979). A smaller but also substantial peak in numbers was in March 1980. Mid-winter and mid-summer numbers of polychaetes were low. A greater number of polychaetes occurred on bricks at the MLLW tide level. Mean difference due to tide level far exceeded the difference due to oil treatment, although the latter effect was statistically significant.

Analyses of variance were also performed on the numbers of species per group, in total, and by taxonomic group (Table 7). For each of the groups, and as a whole, statistically significant differences in number of species per brick were demonstrated for the effect of month of experimentation, effect due to tide level, and effect due to oiling.

The mean numbers of species per brick are graphically displayed by month of experiment in Figure 6. Total species peaked in October 1979, March 1980, and May 1980, and were closely paralleled by crustacean species, the major contributor (Figure 6). Polychaetes also followed the same trend with the exception that the winter decline ended by December 1979, as compared to January for crustaceans and total species. Numbers of mollusk species were lower than for the other taxonomic groups, and the maximum number of species occurred in June 1980. The mollusks did have peaks in October 1979 and March 1980 which paralleled the numbers of species for other groups.

A comparison of mean numbers of species per brick between the two tide levels is shown in Figure 7. The MLLW tide level exhibited a greater number of species per brick for all groups with the exception of mollusks. A similar type comparison of mean number of species between oil-treated and untreated bricks is in Figure 8. For each of the taxonomic categories a greater number of species per brick is indicated for the control bricks. These differences, without exception, were statistically significant. In an attempt to evaluate whether or not the trophic mode of species related to oil treatment effects, the species were grouped as: (1) suspension-

Table 5. Abbreviated analysis of variance for density (individuals per brick) of *Exosphaeroma* sp.

SOURCES	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[1]
Month	9	6938.7	Yes
Tide	1	19773.0	Yes
Oil	1	1264.5	No
Error	550	246.56	

[1] Probability for Type I error is equal to or less than 0.01.

Table 6. Species composition and analysis of variance for polychaete density in monthly hard substrate recovery experiments at Sequim Bay.

COMPOSITION	
SPECIES	TROPHIC CATEGORY[1]
Anatides groenlandica	carnivore
Anatides sp.	carnivore
Armandia brevis	detritivore
Armandia sp.	detritivore
Cirratulus c. cirratus	detritivore
Eulalia sp.	carnivore
Exogone verrugera	detritivore
Exogone sp.	detritivore
Halosynda brevisetosa	carnivore
Harmothoe imbricata	carnivore
Harmothoe sp.	carnivore
Nereis vexillosa	herbivore
Nothria elegans	herbivore
Ophiodromus pugettensis	carnivore
Platynereis bicanaliculata	herbivore
Polydora socialis	detritivore
Polydora sp.	detritivore
Sphaerosyllis californiensis	detritivore
Spionidae (undet.)	detritivore
Terebellidae (undet.)	detritivore
Thelepus crispus	detritivore

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[1] Trophic classification after Simenstad et al. (1979).

	ANALYSIS OF VARIANCE FOR INDIVIDUALS PER BRICK		
SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[2]
Month	9	72.227	Yes
Tide Level	1	198.45	Yes
Oil Treatment	1	29.05	Yes
Error	550	2.58	

[2] Probability for Type I error is equal to or less than 0.01.

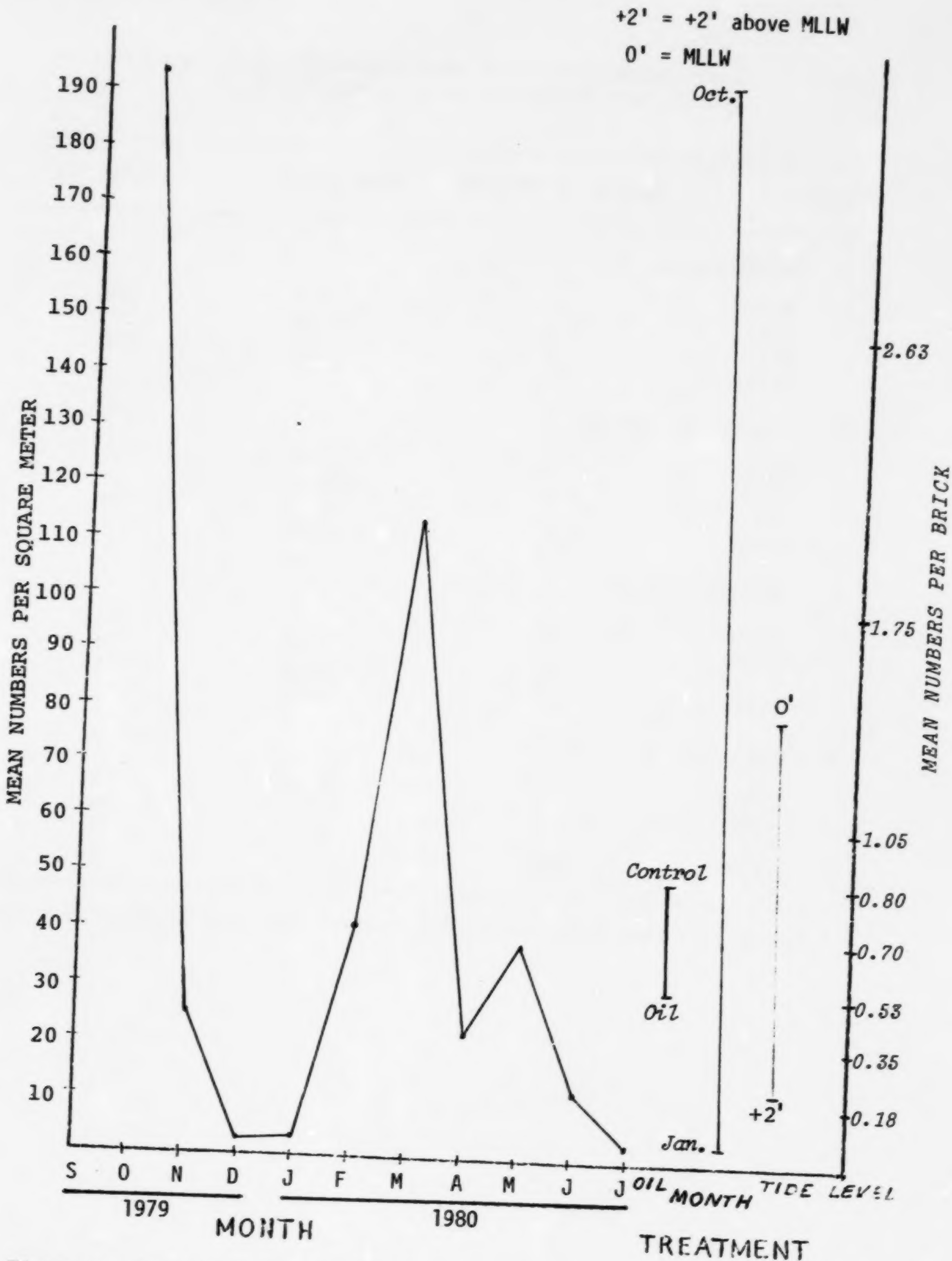

Figure 5. Mean densities for polychaetes in numbers per square meter by month and in numbers per brick by treatment.

Table 7. Analyses of variance of mean number of species per brick in monthly hard substrate recovery experiments.

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[1]
1. Polychaete Species			
Month	9	7.97	Yes
Tide	1	23.36	Yes
Oil	1	6.95	Yes
Error	550	0.25	
2. Crustacean Species			
Month	9	31.69	Yes
Tide	1	106.63	Yes
Oil	1	15.73	Yes
Error	550	0.86	
3. Mollusk Species			
Month	9	2.21	Yes
Tide	1	2.80	Yes
Oil	1	1.78	Yes
Error	550	0.25	
4. Total Species			
Month	9	81.21	Yes
Tide	1	190.18	Yes
Oil	1	62.05	Yes
Error	550	1.71	

[1] Probability that Type I error committed is less than or equal to 0.01.

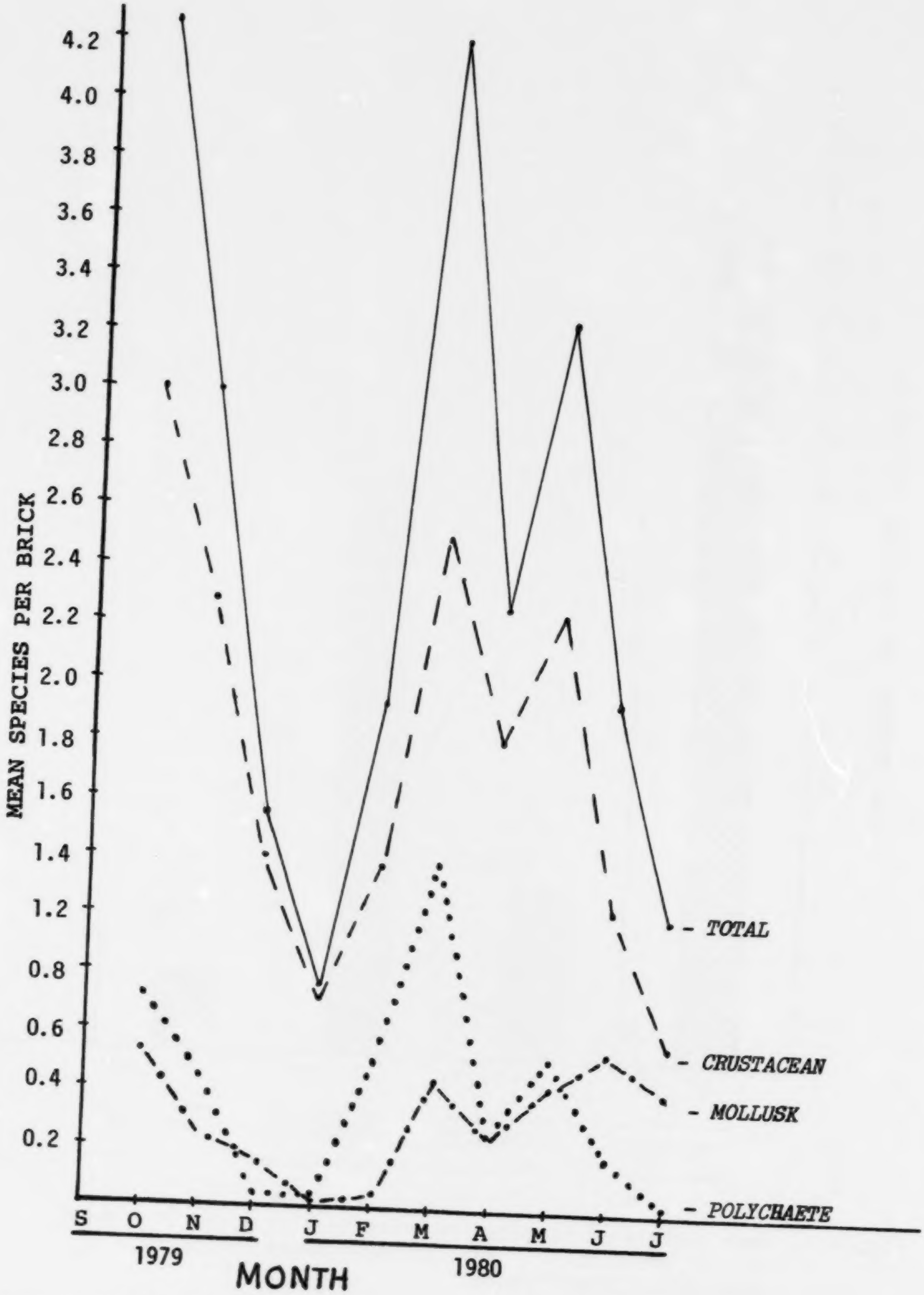

Figure 6. Mean number of species per brick by month for taxonomic groups and total species (data includes both control and oiled bricks).

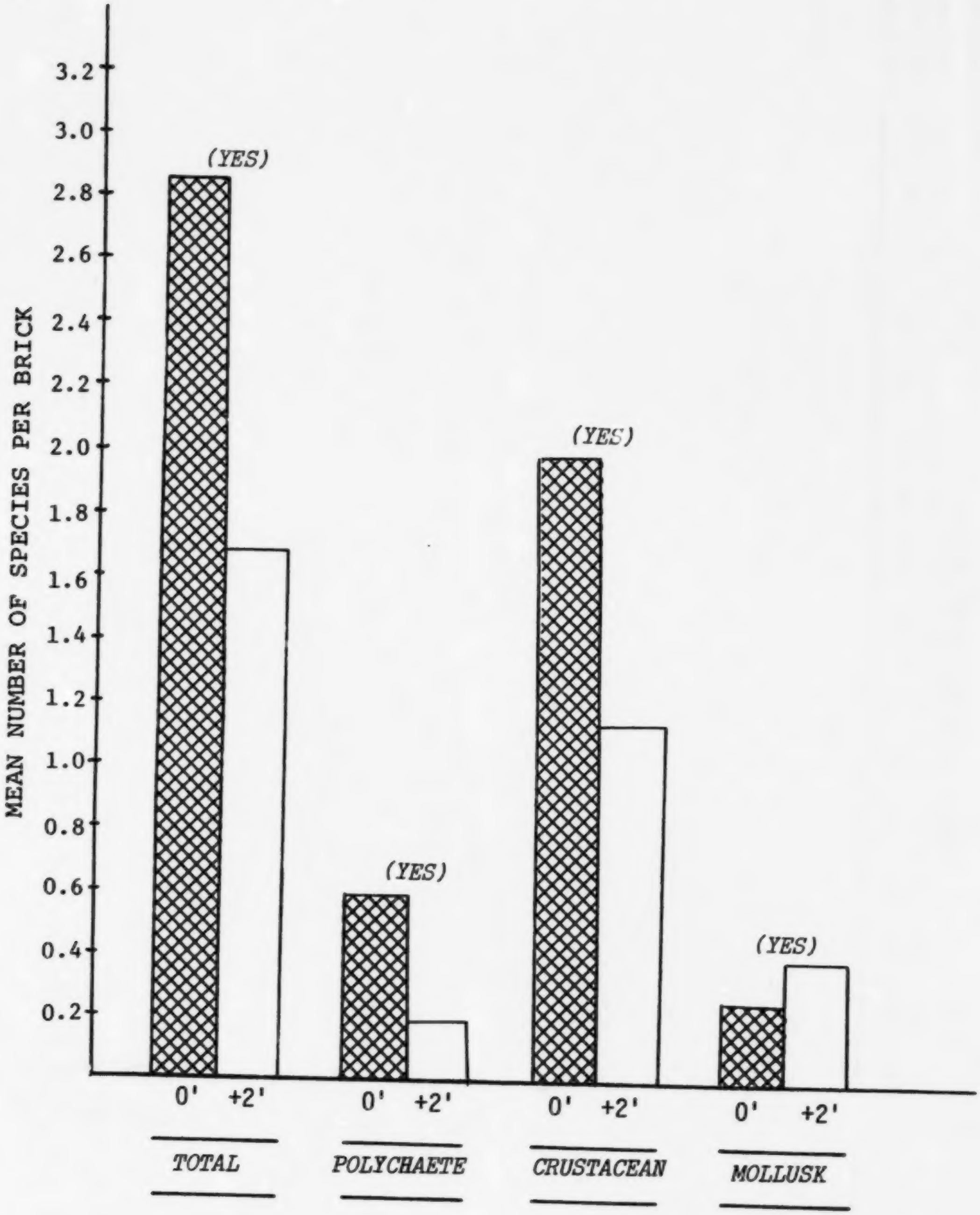

Figure 7. Mean numbers of species per brick by tide level (0 = MLLW; +2 = 2' above MLLW). Word in parentheses indicates statistical significance.

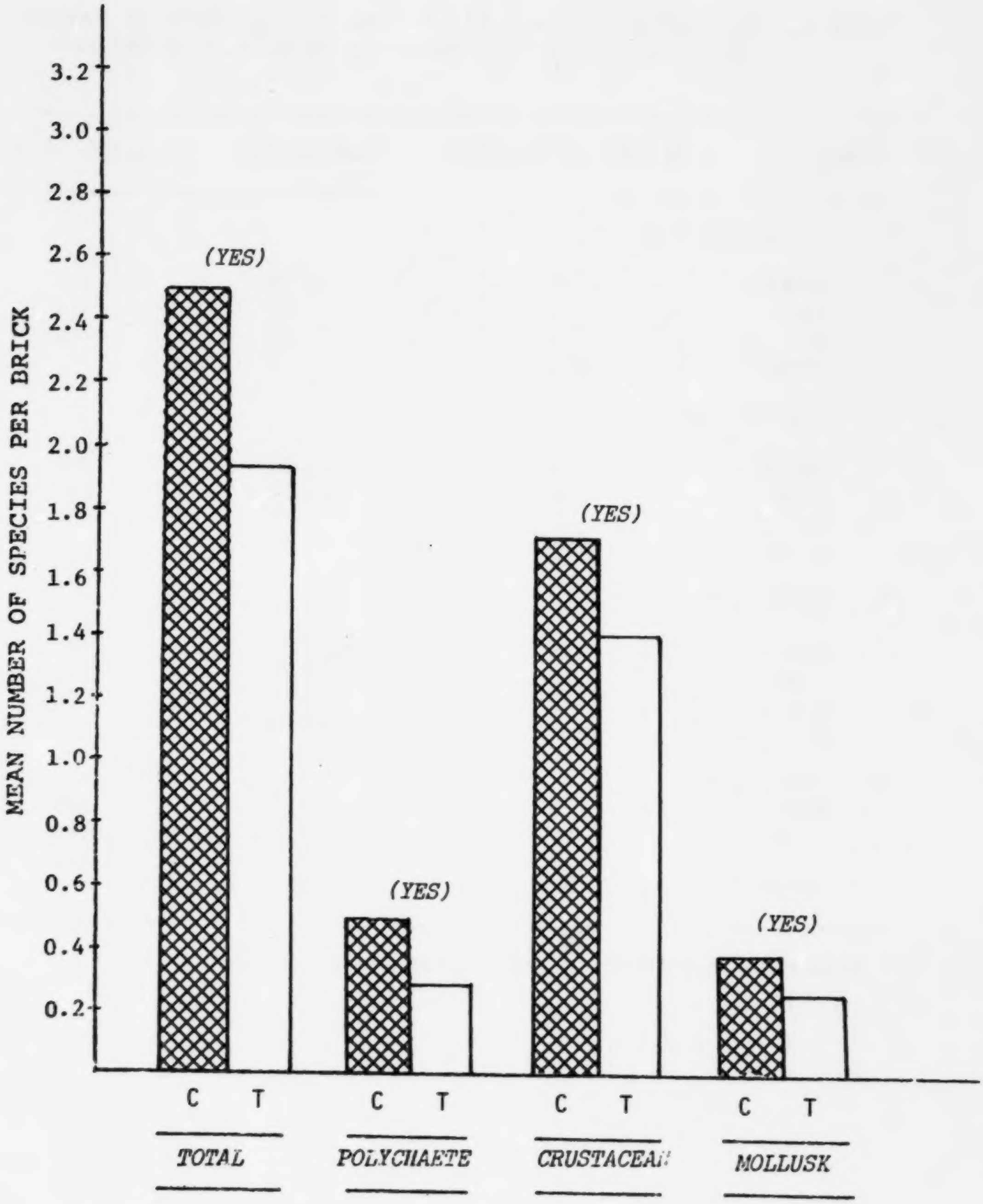

Figure 8. Mean numbers of species per brick by oil treatment (C = control; T = oil treated). Word in parentheses indicates statistical significance.

Table 8. Abbreviated analyses of variance for densities of trophic groups in monthly hard substrate recovery experiments.

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[1]
1. Suspension Feeders			
Month	9	4.98	Yes
Tide	1	7.25	Yes
Oil	1	9.26	Yes
Error	550	0.70	
2. Detritivores			
Month	9	955.97	Yes
Tide	1	885.00	Yes
Oil	1	6.00	No
Error	550	28.92	
3. Herbivores			
Month	9	8010.9	Yes
Tide	1	5342.8	Yes
Oil	1	1631.5	No
Error	550	265.26	
4. Carnivores			
Month	9	6.31	Yes
Tide	1	15.03	Yes
Oil	1	7.56	Yes
Error	550	0.76	

---

[1] Reject the hypothesis of zero effect at $p = 0.01$.

feeders; (2) detritivores; (3) herbivores; and (4) carnivores. Only two of the 79 species were not accommodated by this grouping. An analysis of variance was computed for the density per brick for each of the groups, and abbreviated tables are shown in Table 8. Statistically significant ($p = 0.01$) effects on trophic group density from tide level and month were demonstrated for every group. Statistically significant effects from the oil treatment were demonstrated for suspension-feeders and carnivores.

The mean densities of suspension-feeders by month and treatment are shown in Figure 9. Peak density for suspension-feeders occurred in June at the time of mollusk peak density (Figure 2). The statistically significant effect of oil treatment on suspension-feeders was about equal to tide level effects. A greater number of suspension-feeders was at the +2' tide level. Effects from month of experiment were much greater than tide level or oil treatment effects.

Mean densities for detritivores are shown in Figure 10. Peak density for this group was in the October 1979 experiment. A secondary peak in the March 1980 experiment is much lower than that in October. The difference in mean density indicated between control and oil-treated bricks (C-O, Figure 10) is negligible. In contrast to the suspension-feeders, a greater number of detritivores was at the MLLW tide level.

The mean density for herbivores is shown in Figure 11. Peak density was in the October 1979 experiment, and this peak was about twice the secondary peak in the March 1980 experiment. The difference in density due to oil treatment was not statistically significant. A greater density for herbivores was at the MLLW tide level.

Mean number of carnivores is shown in Figure 12. Peak density was in the March experiment, and a secondary peak was in the May experiment. A greater number of carnivores were at the MLLW tide level. The statistically significant effect on carnivore density due to oil treatment was nearly as large as the effect indicated for tide level.

Site Experiments. Two additional experiments were conducted at Rocky Point during the months of June and July 1980 to evaluate the effect of field exposure site on epifaunal recovery. We attempted to make the Rocky Point experiments identical in all respects to the Sequim Bay "monthly" experiments conducted during those same months. For analyses of data we used the abbreviated model:

$$Y_{ijkl} = S_i + M_j + O_k + T_l + E$$

where Y = magnitude of the response variable;

S = the main effect of site on the response variable; i = (1 = Sequim Bay or 2 = Rocky Point);

M = the main effect of month on the response variable; j = (1 = June or 2 = July 1980);

O = the main effect of oil treatment on the response variable; k = (1 = oiled or 2 = unoiled);

T = the main effect of tide level on the response variable; 1 = MLLW or 2 = +2' above MLLW; and

E = random error.

The response variables evaluated in the model were the same ones evaluated in the monthly experiments, i.e., numbers of individuals per brick and numbers of specific entities per brick. These latter are referred to as "species" with the same qualifications previously given.

A few points of clarification are warranted. First, we are testing hypotheses concerning differences due to site. The data for the Sequim Bay site is the same data used in "monthly" experiment analyses for the months of June and July 1980. For rigorous statistical treatment, these data should not again be used in the present analysis. However, there is no reason to suspect that the use of these data will in any way bias the outcome of the present analysis. Second, the evaluation of the main effect of month in these experiments involves only two months, i.e., June and July 1980. Because of this, differences in main effect means between months can be expected to be much smaller than differences shown over the 10 monthly experiments. Also, means for a response variable magnitude for a given month (June or July, 1980) will be different than those reported under monthly experiments because both sites are used in arriving at these means.

By way of orientation, it is instructive to reexamine Figure 6, which presents numbers of species within taxonomic groups by month of experiment at Sequim Bay. These data indicate that the months chosen for these site comparisons (June and July, 1980) were months in which the total number of species and number of species within the constituent groups (with the exception of mollusks) were at near minimal values. Thus, in retrospect, one can appreciate that peak recovery months (March and October) might have been better for the site comparison.

The sites of experimentation are compared in terms of density within taxonomic and trophic groups in Figure 13. Sequim Bay had a greater number of polychaetes, mollusks, carnivores, and suspension-feeders, while Rocky Point had greater numbers of crustaceans, detritivores, and herbivores. In all cases, with the exception of mollusks and herbivores, the differences in density attributable to site are statistically significant (Table 9, Figure 13). For taxonomic groups, crustaceans contributed the greatest number of individuals per brick at each of the sites. For trophic groups, detritivores were the predominant group at Rocky Point, while herbivores contributed the greatest number of individuals per brick at Sequim Bay.

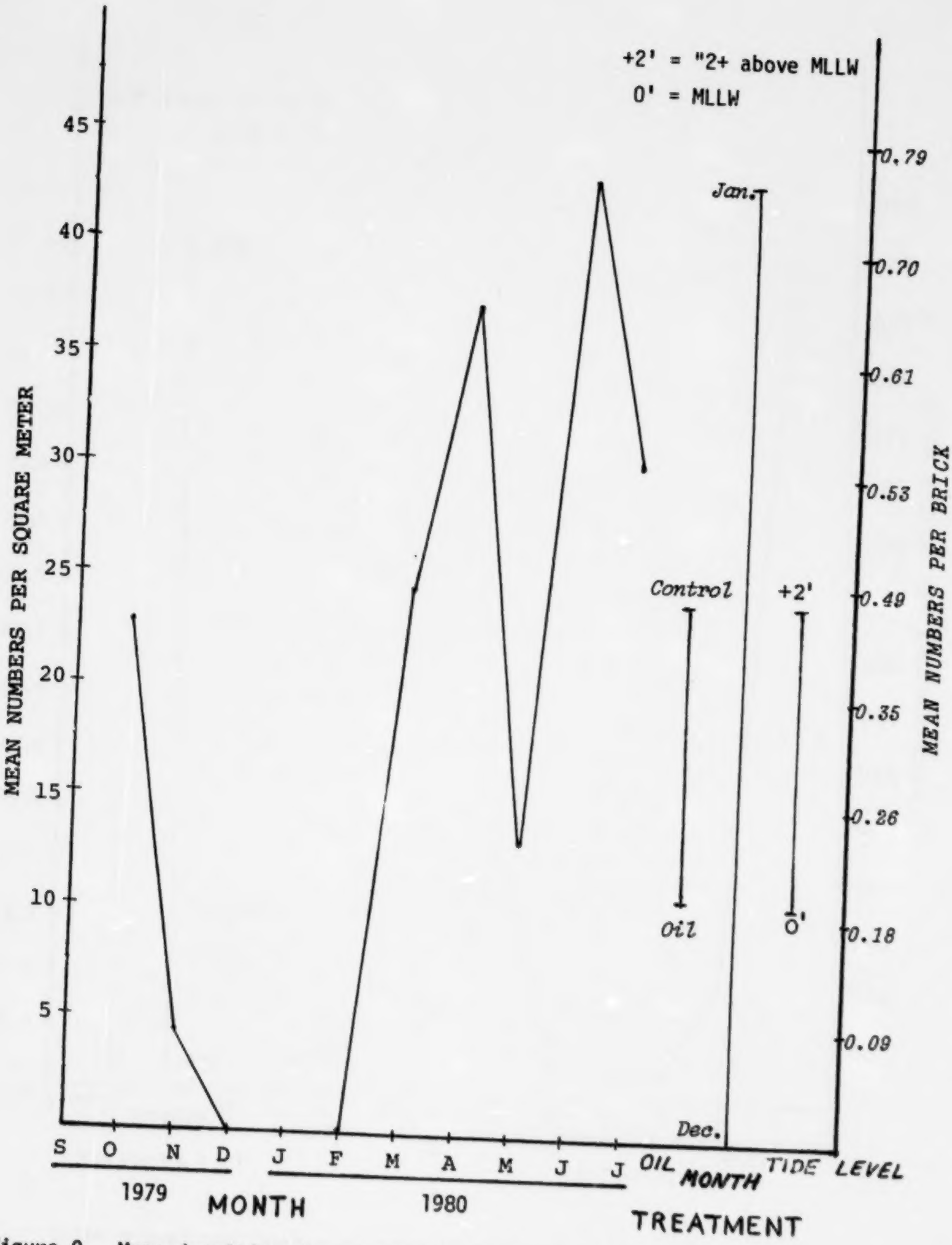

Figure 9. Mean densities for suspension feeders per square meter by month and in numbers per brick per treatment.

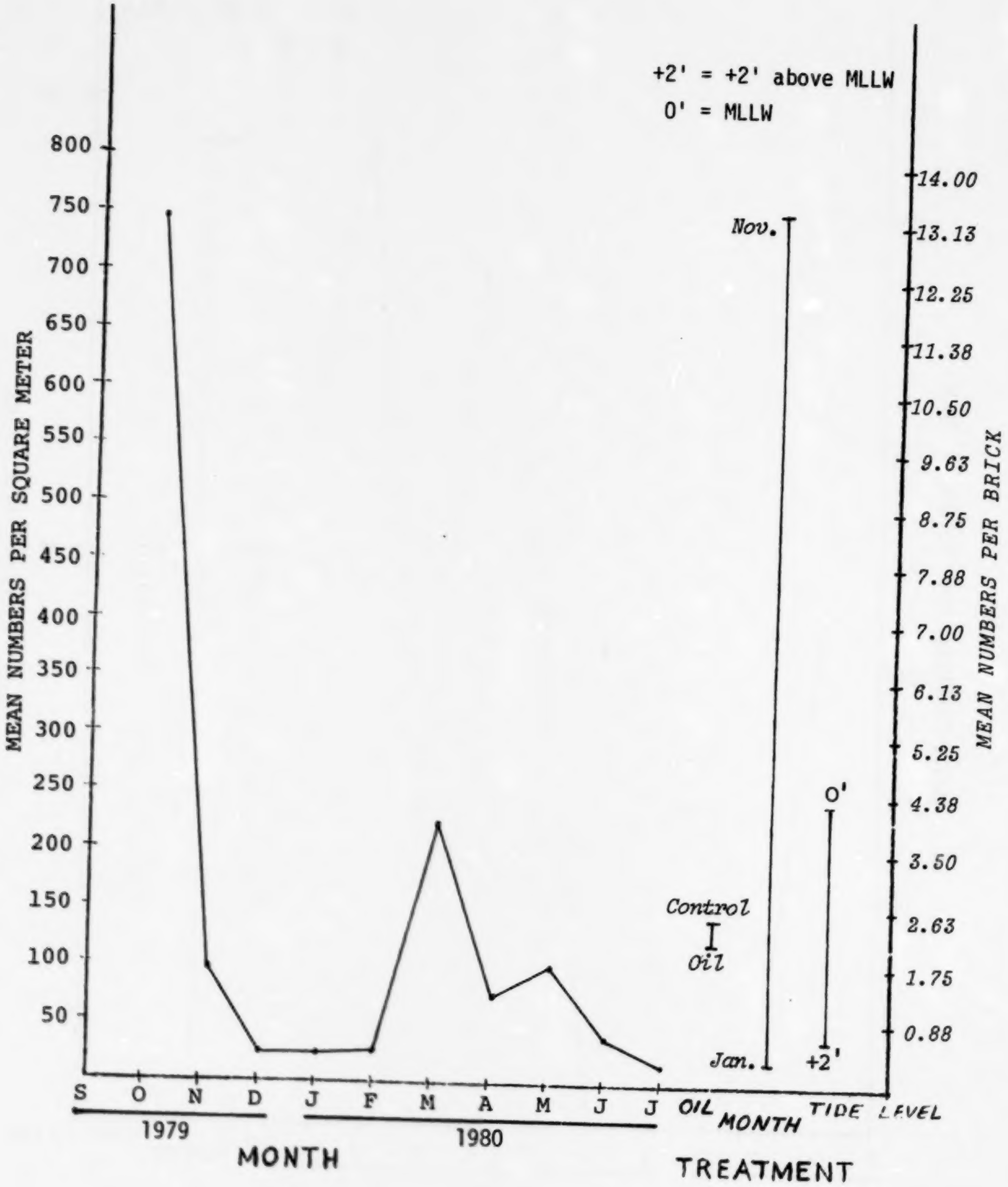

Figure 10. Mean densities for detritivores in numbers per square meter by month and in numbers per brick by treatment.

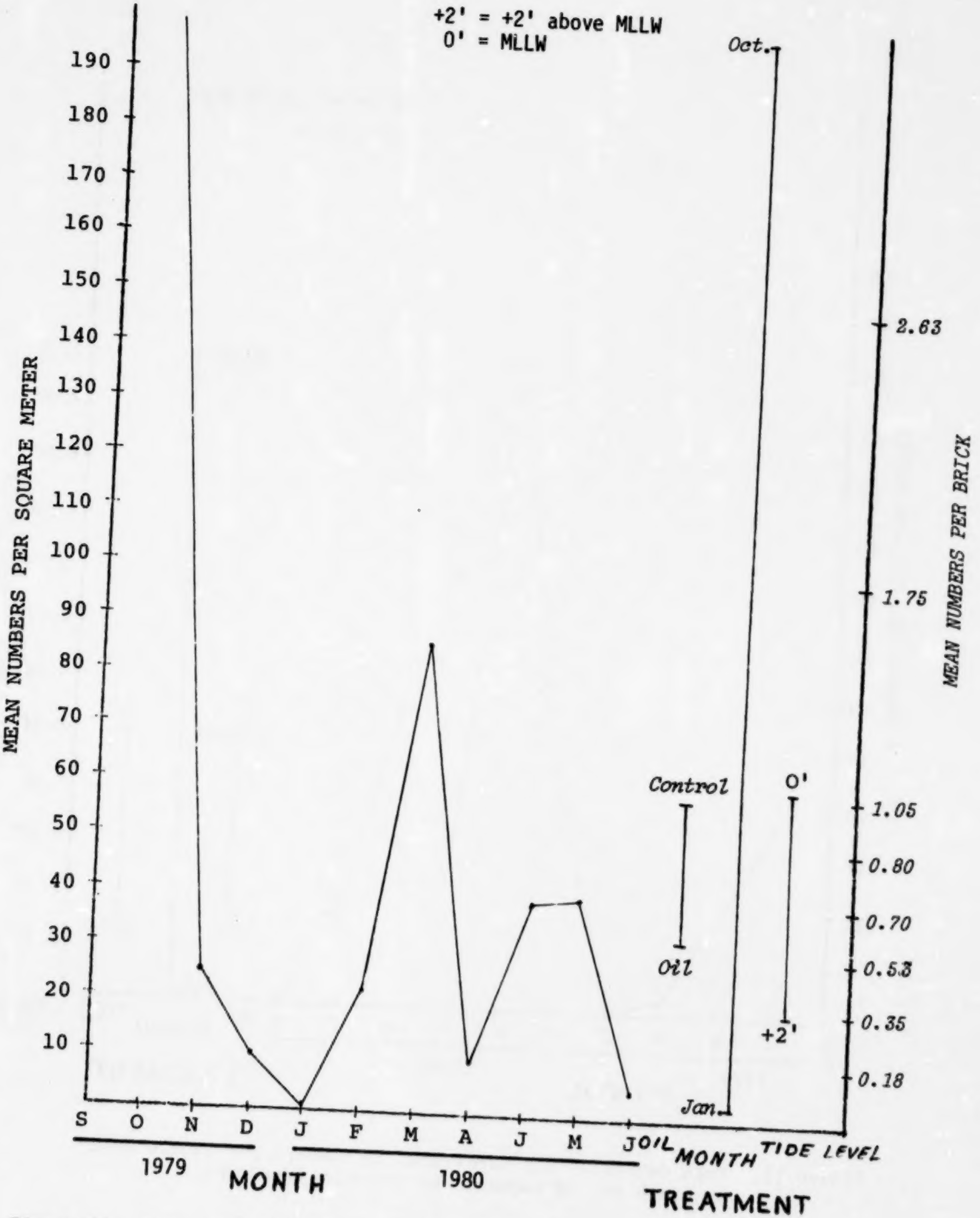

Figure 11. Mean densities for herbivores in numbers per square meter by month and in numbers per brick by treatment.

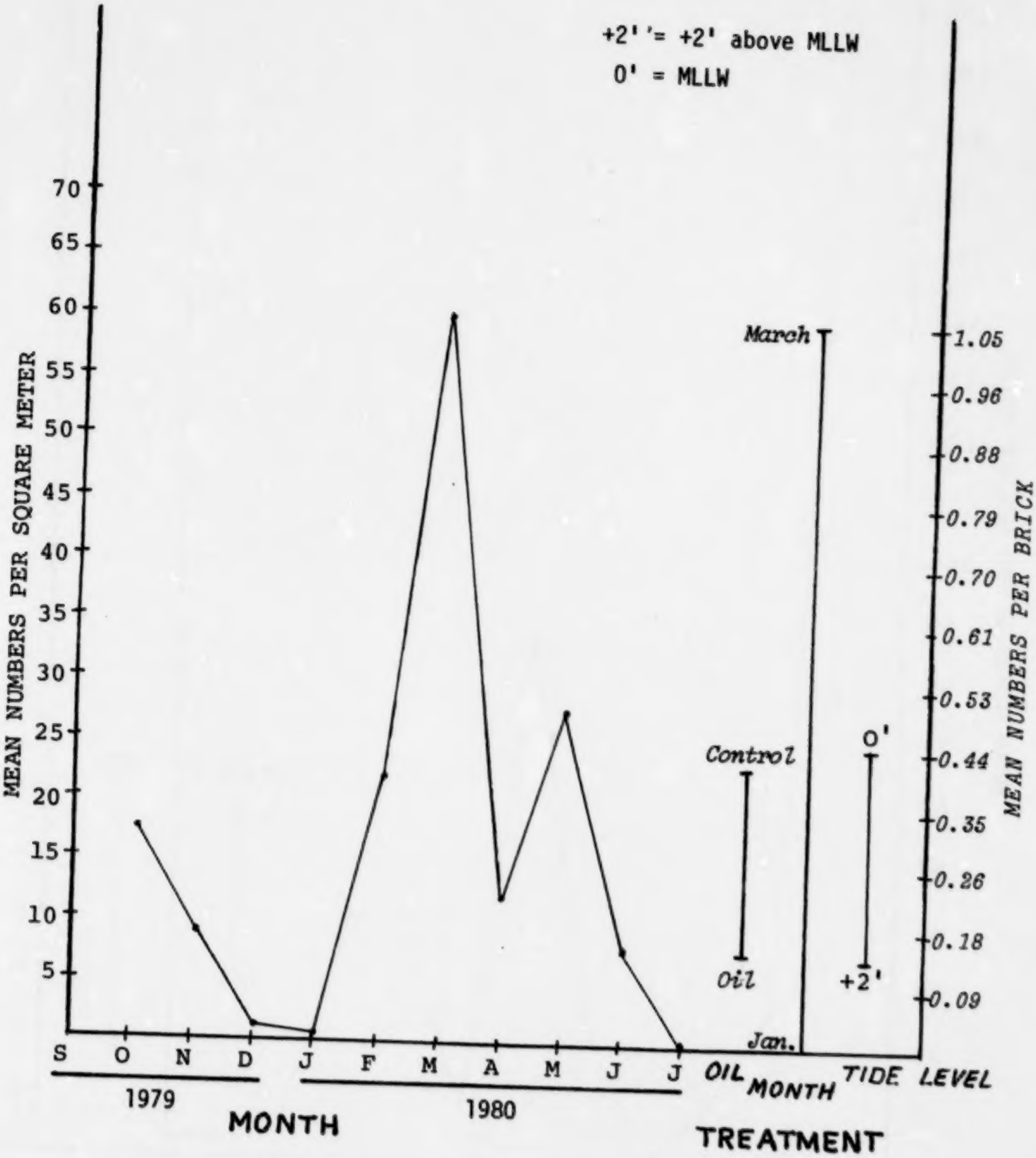

Figure 12. Mean densities for carnivores in numbers per square meter by month and in numbers per brick by treatment.

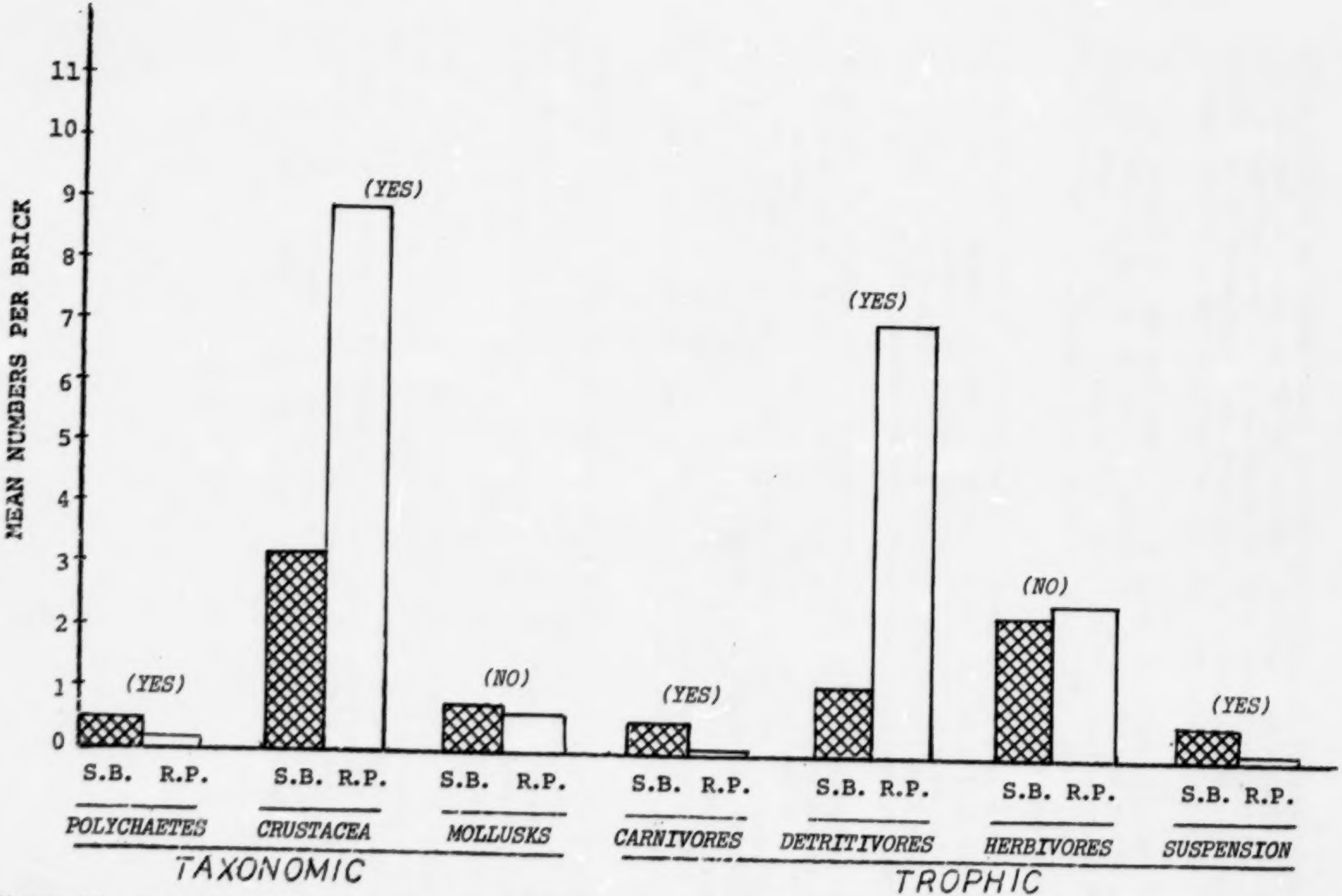

Figure 13. Mean densities of individuals within taxonomic and trophic groups by site (S.B. = Sequim Bay; R.P. = Rocky Point). Data summarized over two tide levels (MLLW and +2' above MLLW); two oil treatments (oiled and unoiled); and two months (June and July 1980). Word in parentheses indicates statistical significance.

A comparison of taxonomic and trophic group densities between months is shown in Figure 14. Greater numbers per brick were in June for all taxonomic and trophic groups with the exception of suspension-feeders. The differences due to the month of experiment were statistically significant (Table 9, Figure 14) with the exception of mollusks and suspension-feeders.

Tide level effects on group density are compared in Figure 15. All groups with the exception of suspension-feeders exhibited a higher density at the MLLW tide level. Tide level differences in density were statistically significant (Table 9, Figure 15) except for mollusks and suspension feeders.

The effect of oil treatment on group density in the site experiments is presented in Figure 16. In all cases, a smaller number of individuals per brick is observable on oil-treated bricks as compared to controls. Statistically significant effects from the oil treatment were demonstrated for polychaetes, mollusks, carnivores, and herbivores (Table 9, Figure 16).

The influence of site and oil treatment on the numbers of species per brick in taxonomic groups for the site experiments is shown in Table 10 and Figure 17. A comparison of sites (Figure 17A) reveals a greater total number of species per brick and greater numbers of polychaete and crustacean species at Sequim Bay than at Rocky Point. These indicated site differences are statistically significant (Table 10 and Figure 17). A greater mean number of species of mollusks per brick is shown for Rocky Point (Figure 17), but the difference is not statistically significant (Table 10).

Numbers of species per brick for polychaetes, crustaceans, mollusks, and overall, are shown in Figure 17B as related to oil treatment. In every instance a greater number of species is shown for control bricks as compared to oil-treated ones. The differences indicated are statistically significant (Table 10, Figure 17) in every instance.

Numbers of species per brick comparisons of month and tide level effects are shown in Figure 18. Total number of species per brick, as well as numbers of species in each of the constituent groups, was higher in June than in July. Differences attributable to month of experiment were statistically significant (Table 10, Figure 18) except for mollusks. There was a larger number of species at the MLLW tide level as compared to the +2' tide level in every instance. For mollusks, the tide level effect was not significant.

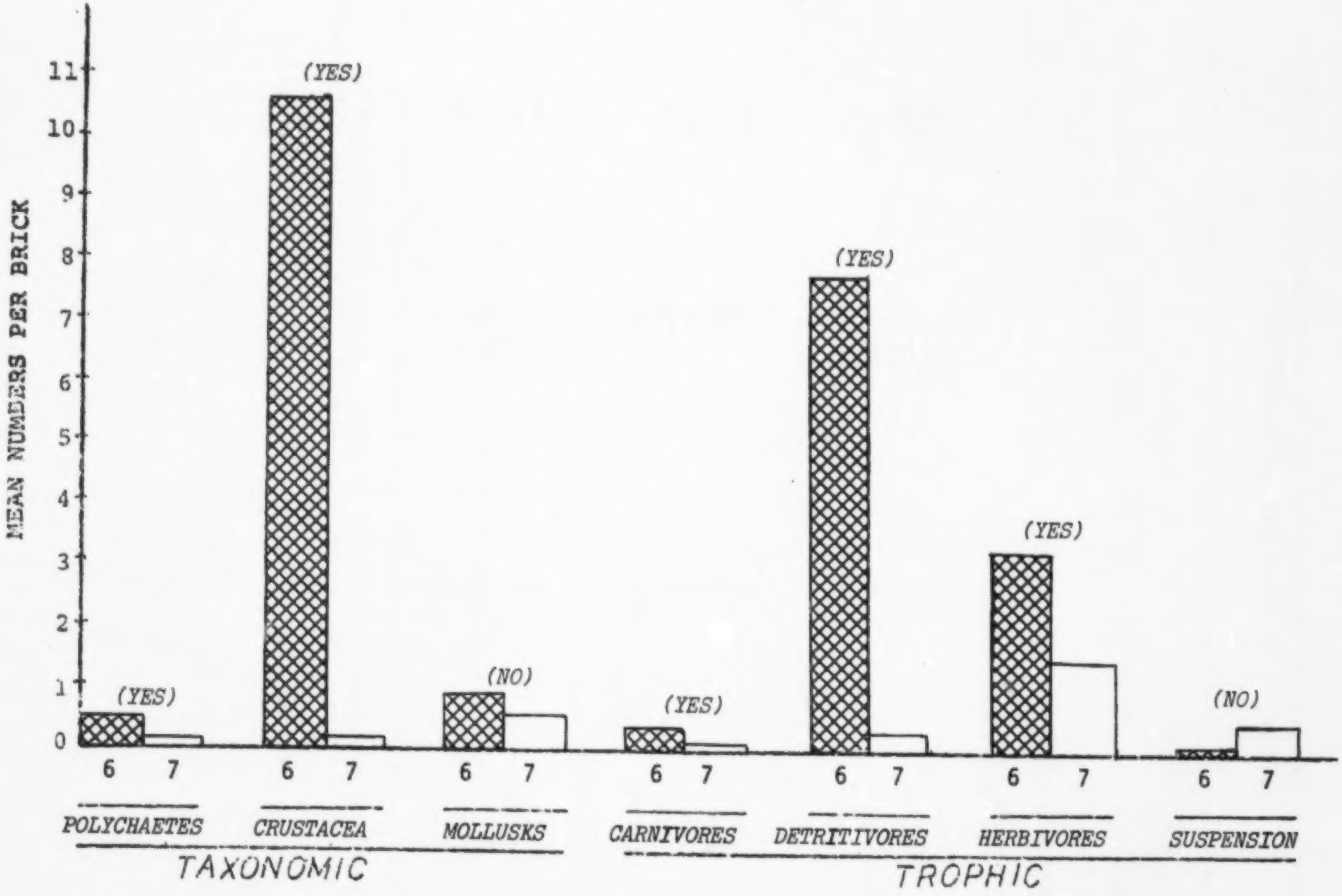

Figure 14. Mean densities of individuals within taxonomic and trophic groups by month in site experiment (6 = May; 7 = June). Data summarized over two sites (Rocky Point and Sequim Bay); two tide levels (MLLW and +2 above MLLW); and two months (June and July 1980). Word in parentheses indicates statistical significance.

Table 9. Analyses of variance for density of taxonomic and trophic groups in hard substrate site experiment.[1]

RESPONSE VARIABLE/ SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[2]
TAXONOMIC GROUPS			
1. Crustaceans			
Site	1	1991.5	Yes
Month	1	4543.5	Yes
Oil	1	233.9	No
Tide	1	4591.2	Yes
Error	220	68.44	
2. Polychaetes			
Site	1	5.37	Yes
Month	1	8.89	Yes
Oil	1	5.11	Yes
Tide	1	6.96	Yes
Error	220	0.31	
3. Mollusks			
Site	1	0.31	No
Month	1	8.72	No
Oil	1	18.06	Yes
Tide	1	2.94	No
Error	220	1.64	

---

[1] Analyses of variance performed on data from two sites (Sequim Bay and Rocky Point); two months (June and July 1980); and two tide levels (MLLW and +2' above MLLW). The densities of organisms on hard substrates were those which resulted after a 30-day field colonization period.

[2] Probability for Type I error equal to or less than 0.01.

Table 9. (Continued)

RESPONSE VARIABLE/ SOURCE OF OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[2]
TROPHIC GROUPS			
1. Carnivores			
Site	1	3.60	Yes
Month	1	2.95	Yes
Oil	1	3.29	Yes
Tide	1	2.84	Yes
Error	220	0.26	
2. Detritivores			
Site	1	2116.3	Yes
Month	1	3530.1	Yes
Oil	1	44.2	No
Tide	1	2759.0	Yes
Error	220	53.9	
3. Herbivores			
Site	1	3.12	No
Month	1	180.14	Yes
Oil	1	125.25	Yes
Tide	1	401.03	Yes
Error	220	11.87	
4. Suspension-Feeders			
Site	1	11.32	Yes
Month	1	3.51	No
Oil	1	3.12	No
Tide	1	5.05	No
Error	220	0.95	

---

[1] Analyses of variance performed on data from two sites (Sequim Bay and Rocky Point); two months (June and July 1980); and two tide levels (MLLW and +2' above MLLW). The densities of organisms on hard substrates were those which resulted after a 30-day field colonization period.

[2] Probability for Type I error equal to or less than 0.01.

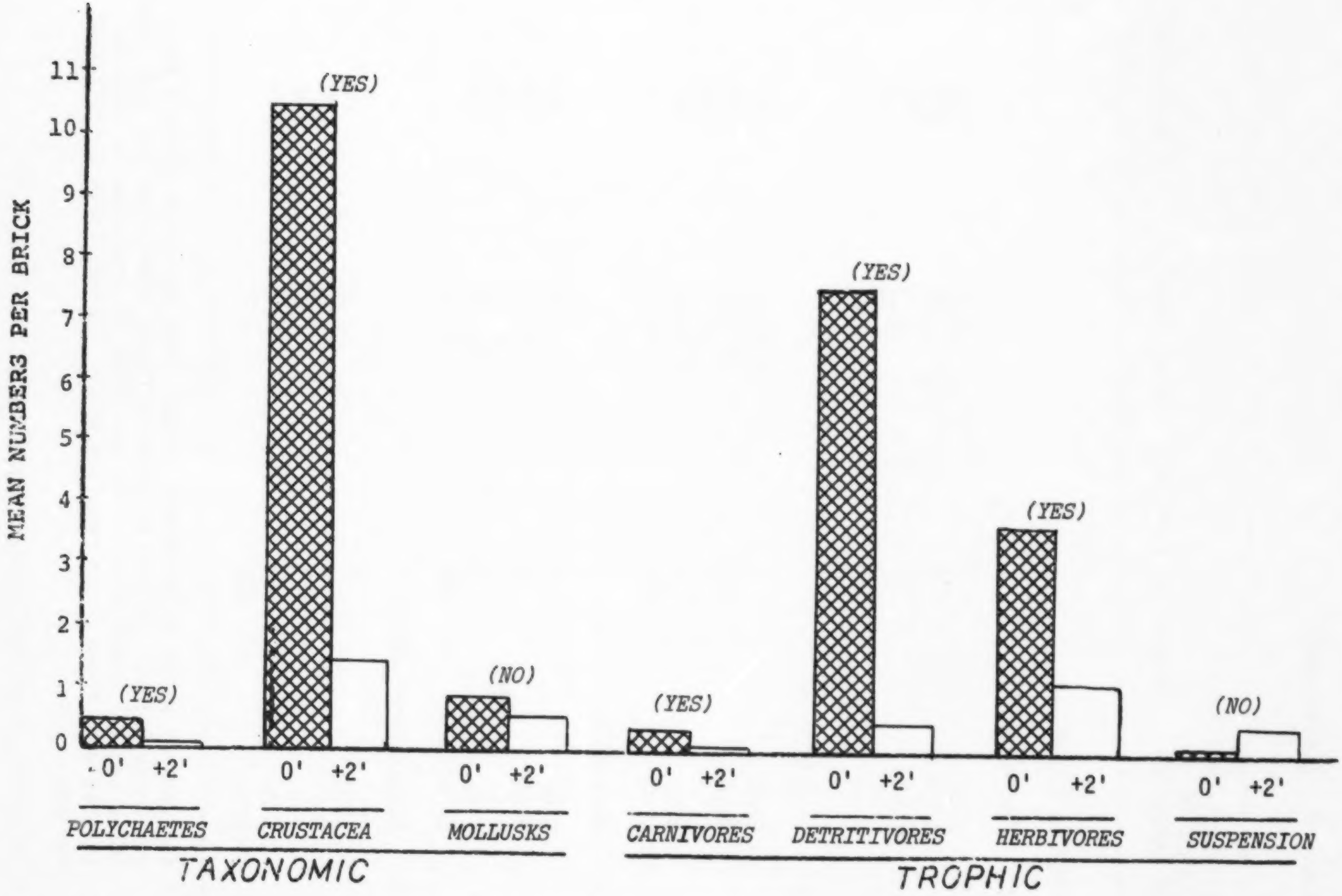

Figure 15. Mean densities of individuals within taxonomic and trophic groups by tide level (0' = MLLW; +2 = 2' above MLLW). Word in parentheses indicates statistical significance. Data summarized over two sites (Rocky Point and Sequim Bay); two months (June and July 1980); and two oil treatments (oiled and unoiled).

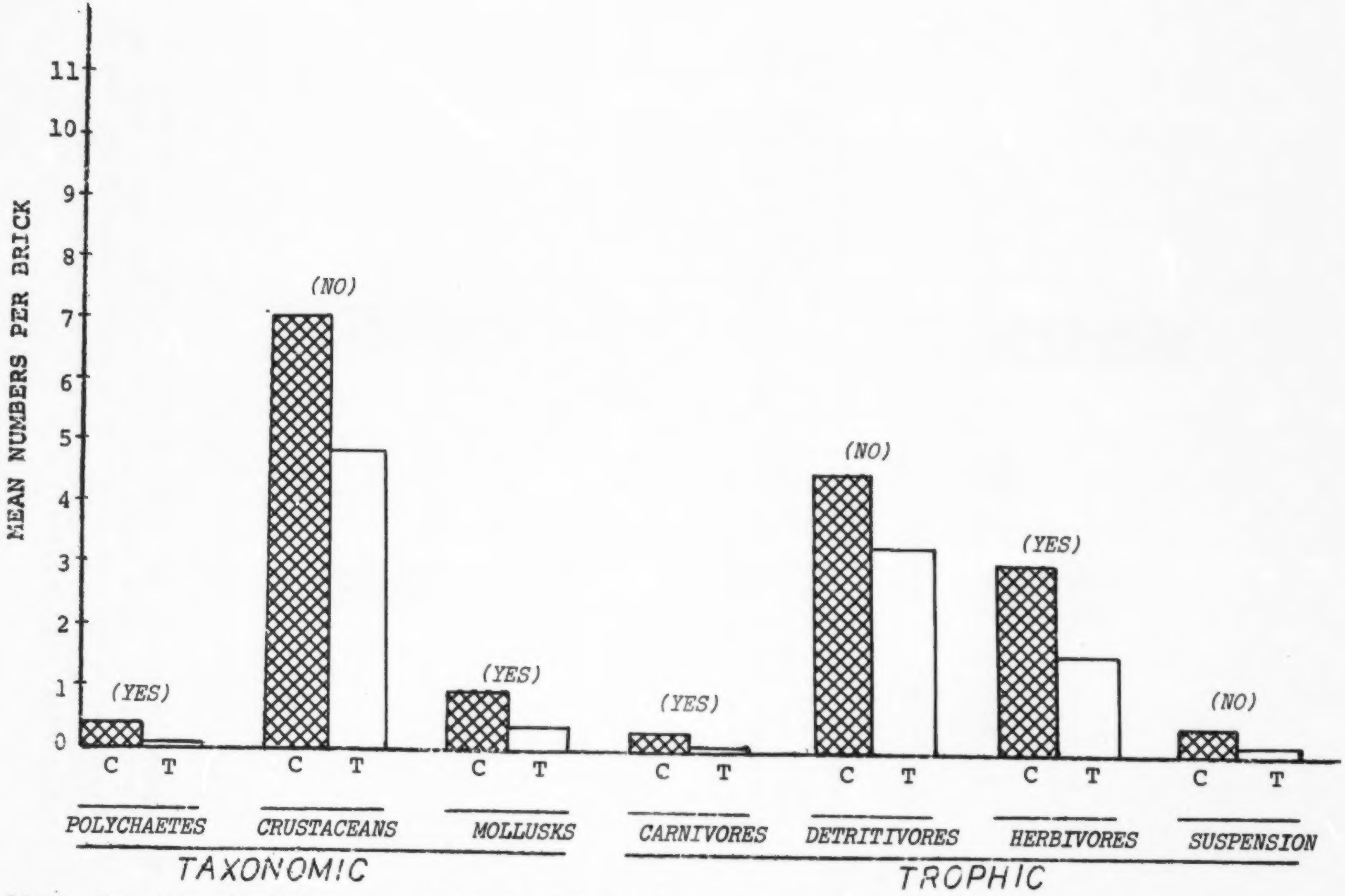

Figure 16. Mean densities of individuals within taxonomic and trophic groups by oil treatment (C = control; T = oil). Data summarized over two sites (Rocky Point and Sequim Bay); two months (June and July 1980); and two tide levels (MLLW and +2' above MLLW). A 30-day field exposure period was used. Word in parentheses indicates statistical significance.

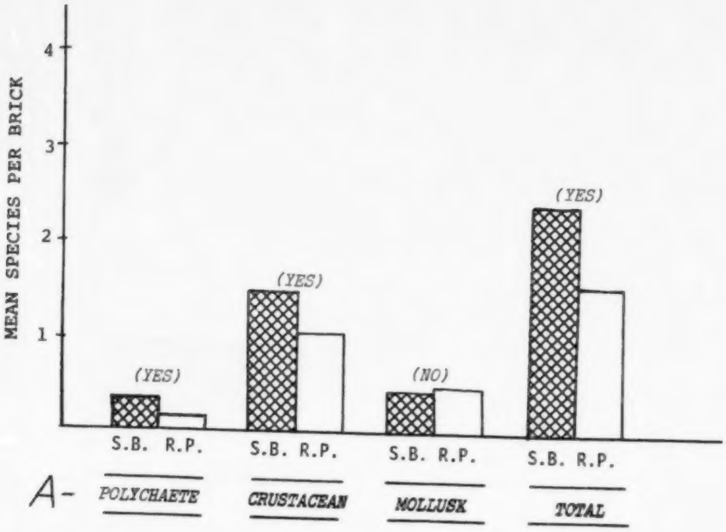

Figure 17A. Number of species per brick by site (S.B. = Sequim Bay; R.P. = Rocky Point). Data summarized over two months (June and July 1980); two tide levels (MLLW and +2' above MLLW); and two oil treatments (oiled and unoiled). Word in parentheses indicates statistical significance.

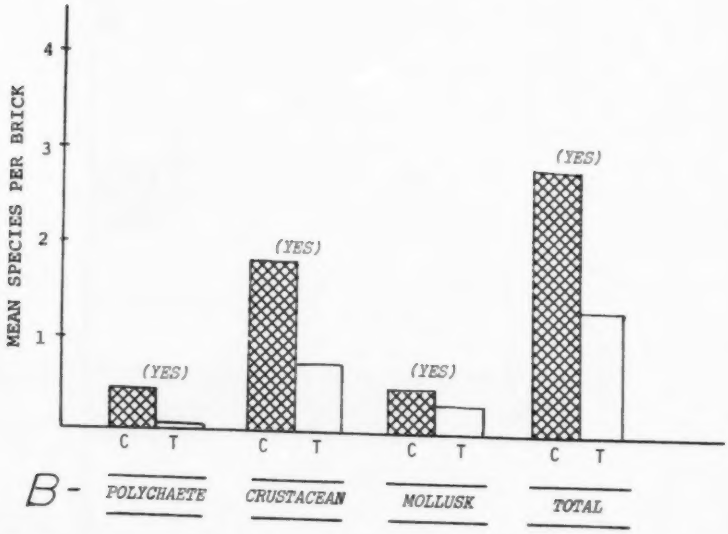

Figure 17B. Number of species per brick by oil treatment (C = control; T = treated). Data summarized over two sites (Rocky Point and Sequim Bay); two tide levels (MLLW and +2' above MLLW); and two months (June and July 1980). Word in parentheses indicates statistical significance.

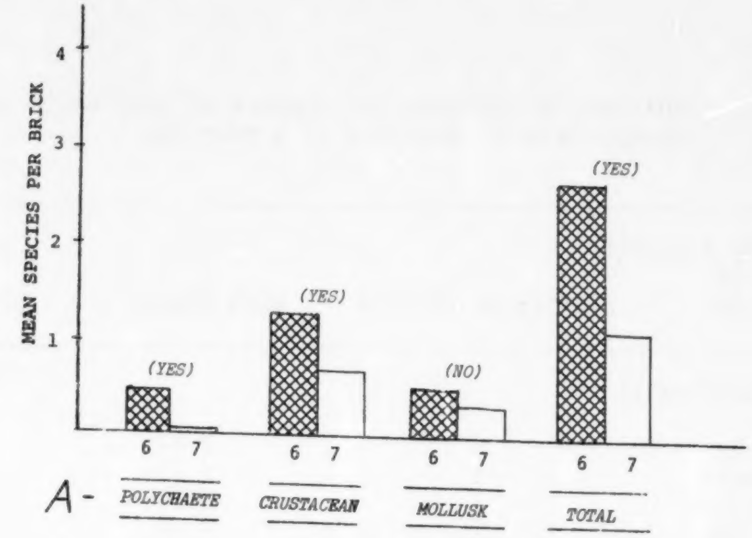

Figure 18A. Number of species per brick by month (6 = June; 7 = July). Data summarized over two sites (Rocky Point and Sequim Bay); two tide levels (MLLW and +2' above MLLW); and two treatments (control and treated). Word in parentheses indicates statistical significance.

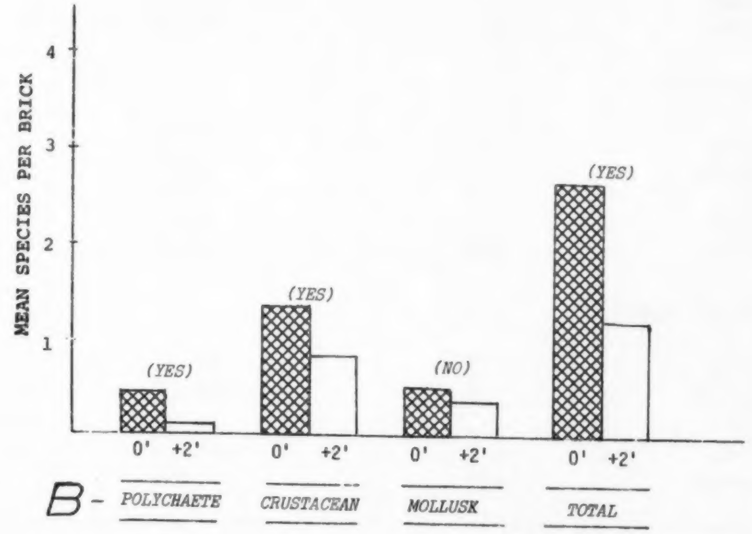

Figure 18B. Number of species per brick by tide level (+2' = 2' above MLLW) after 30-day field exposure. Data summarized over two sites (Rocky Point and Sequim Bay); two months (June and July 1980); and two oil treatments (oiled and unoiled). Word in parentheses indicates statistical significance.

Table 10. Analyses of variance for numbers of species in taxonomic groups in hard substrate site experiments.[1]

RESPONSE VARIABLE/ SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	SIGNIFICANCE[2]
1. Polychaetes			
Site	1	2.61	Yes
Month	1	5.73	Yes
Oil	1	2.81	Yes
Tide	1	4.76	Yes
Error	220	0.19	
2. Crustaceans			
Site	1	15.09	Yes
Month	1	80.14	Yes
Oil	1	6.26	Yes
Tide	1	56.87	Yes
Error	220	0.74	
3. Mollusks			
Site	1	1.38	No
Month	1	1.64	No
Oil	1	2.37	Yes
Tide	1	0.72	No
Error	220	0.28	
4. Total Species			
Site	1	47.11	Yes
Month	1	164.50	Yes
Oil	1	31.93	Yes
Tide	1	118.74	Yes
Error	220	1.59	

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[1] Data included two sites (Rocky Point and Sequim Bay); two months (June and July 1980); two oil treatments (oiled and unoiled); and two tide levels (MLLW and +2' above MLLW). Number of species resulted from a 30-day field exposure.

[2] Probability for Type I error is equal to or less than 0.01.

Hard Substrate Recovery - Total Oil Concentrations.

Monthly Experiments. Infrared analyses for total oil and capillary gas chromatography for selected saturate and aromatic compounds were performed on samples taken immediately after oil treatment, and at five and 30-day intervals after field placement of bricks.

A time course of total oil concentration in the ten monthly experiments at Sequim Bay is in Figure 19. A more detailed presentation of these data is in Table 11. Two types of data are represented on the figure. The extraction of all oil from individual bricks resulted in the data labeled "W," for whole brick, in Figure 19. The extraction of oil from the top, or colonizing surface only, resulted in the data marked "T" in Figure 19. For both types of data, a marked reduction in total oil between samples taken immediately post-treatment, and at five days after field placement is apparent. The 30-day data declined slightly from 5-day data for both whole brick and top surface extractions. For the top surface extractions, the decline was somewhat less than for whole brick extractions between five and 30 days. Initially, the top surface oil content of bricks was slightly more than half (56%) of whole brick content (Table 11, Figure 19). For five and 30-day samples the top surface of bricks has about 20% (18-21%) of whole brick extractions (Table 11). There was no distinct trend in proportion of top surface to whole brick extractions between five and 30 days.

Data on Figure 19 also show a consistent, but not statistically significant (P = 0.05), higher concentration on bricks placed at +2' above MLLW as compared to bricks placed at MLLW.

The data on Table 11 indicate much higher initial oil concentrations in March, April, May, and June experiments than in preceding months and July. These high initial concentrations undoubtedly relate to a lack of control of some aspect of the treatment itself and/or improvements in extraction efficiency noted in an earlier report (Vanderhorst et al., 1980). However, it can be seen from the data in Figure 20 that the high initial concentrations bear little relation to the concentrations of total oil on the top surface of bricks after five days of field exposure. Data in Figure 20 also permit an experiment-to-experiment comparison in total oil concentration on the top surface of bricks among the several treatment categories.

Site Experiments. A comparison of total oil concentration in the two site experiments involving hard substrates is given in Figures 21 and 22. The overall concentrations of total oil are comparable to the data presented previously from monthly experiments at Sequim Bay. From Figure 21, there is no consistent trend in concentration on bricks due to site. At the end of five days of field exposure, the mean concentrations for whole bricks at respective tide levels were slightly higher at Rocky Point than at Sequim Bay. The converse was true at the end of 30 days of field exposure. Concentrations at the MLLW tide level were slightly, but not significantly (P = 0.05), lower than were those at +2' above MLLW. A comparison of mean total oil data by month of site experiment (June or

Table 11. Mean monthly concentrations of oil on bricks (grams/brick) in hard substrate recovery experiments at Sequim Bay.

MONTH	TOTAL OIL (GRAMS/BRICK) N = 5 BRICKS/MEAN					
	INITIAL	5-Day		30-Day		EXTRACT[1]
		+2'	MLLW	+2'	MLLW	
OCTOBER	3.67	-	-	-	0.49	W
	-	-	-	-	-	T
NOVEMBER	4.06	4.80	1.76	2.43	3.41	W
	0.82	0.89	0.49	0.07	0.03	T
DECEMBER	3.09	3.64	1.97	-	-	W
	1.04	0.07	0.07	-	-	T
JANUARY	2.91	2.01	2.86	1.32	1.92	W
	1.60	0.32	0.08	0.32	0.39	T
FEBRUARY	9.98	3.47	3.16	3.10	1.81	W
	5.78	0.45	0.89	0.72	0.65	T
MARCH	15.60	6.70	8.02	3.84	3.42	W
	8.97	0.98	1.38	0.92	0.57	T
APRIL	10.24	2.56	3.36	1.39	2.38	W
	6.80	1.03	0.93	0.38	0.51	T
MAY	16.86	6.19	5.05	5.28	4.62	W
	8.80	1.32	0.96	1.70	0.77	T
JUNE	14.78	4.02	3.18	3.01	1.49	W
	8.19	1.15	0.72	0.53	0.27	T
JULY	6.25	7.73	6.70	5.93	6.50	W
	2.00	1.28	1.22	0.84	0.86	T
OVERALL MEANS						
WHOLE	8.72	4.57	4.01	3.29	2.89	
TOP	4.89	0.83	0.75	0.69	0.51	
STD. DEV. DUE TO MONTH						
WHOLE	5.53	1.94	2.14	1.67	1.83	
TOP	3.50	0.45	0.46	0.50	0.27	
% RATIO TOP/WHOLE	56	18	19	21	19	

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[1] W = whole brick extraction; T = top surface of brick extraction.

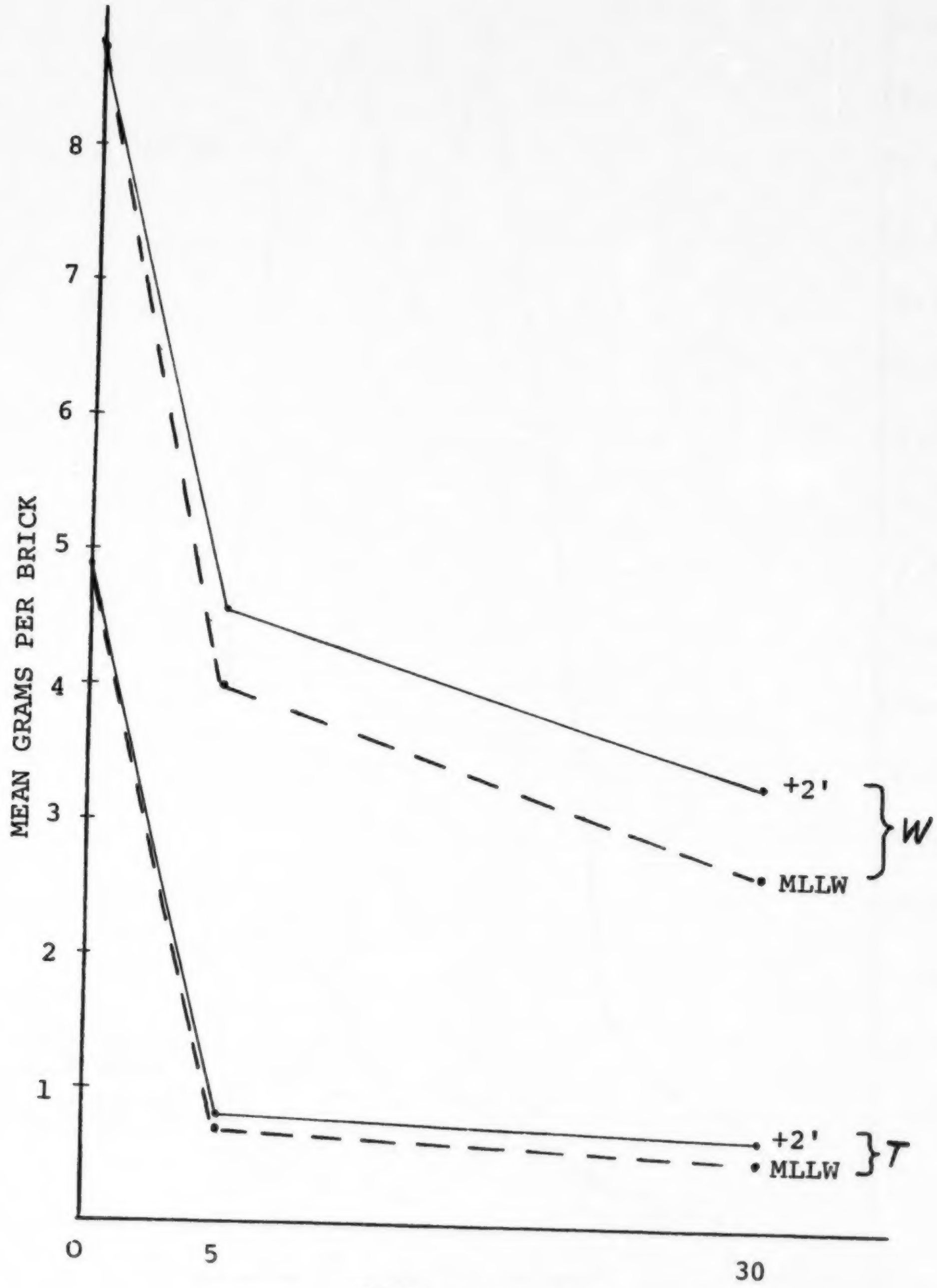

Figure 19. Summary of infrared analyses (IR) in terms of individual experiment time frames (N = 50 measurements per point) in monthly experiments at Sequim Bay (W = whole brick extractions; T = top surface extractions).

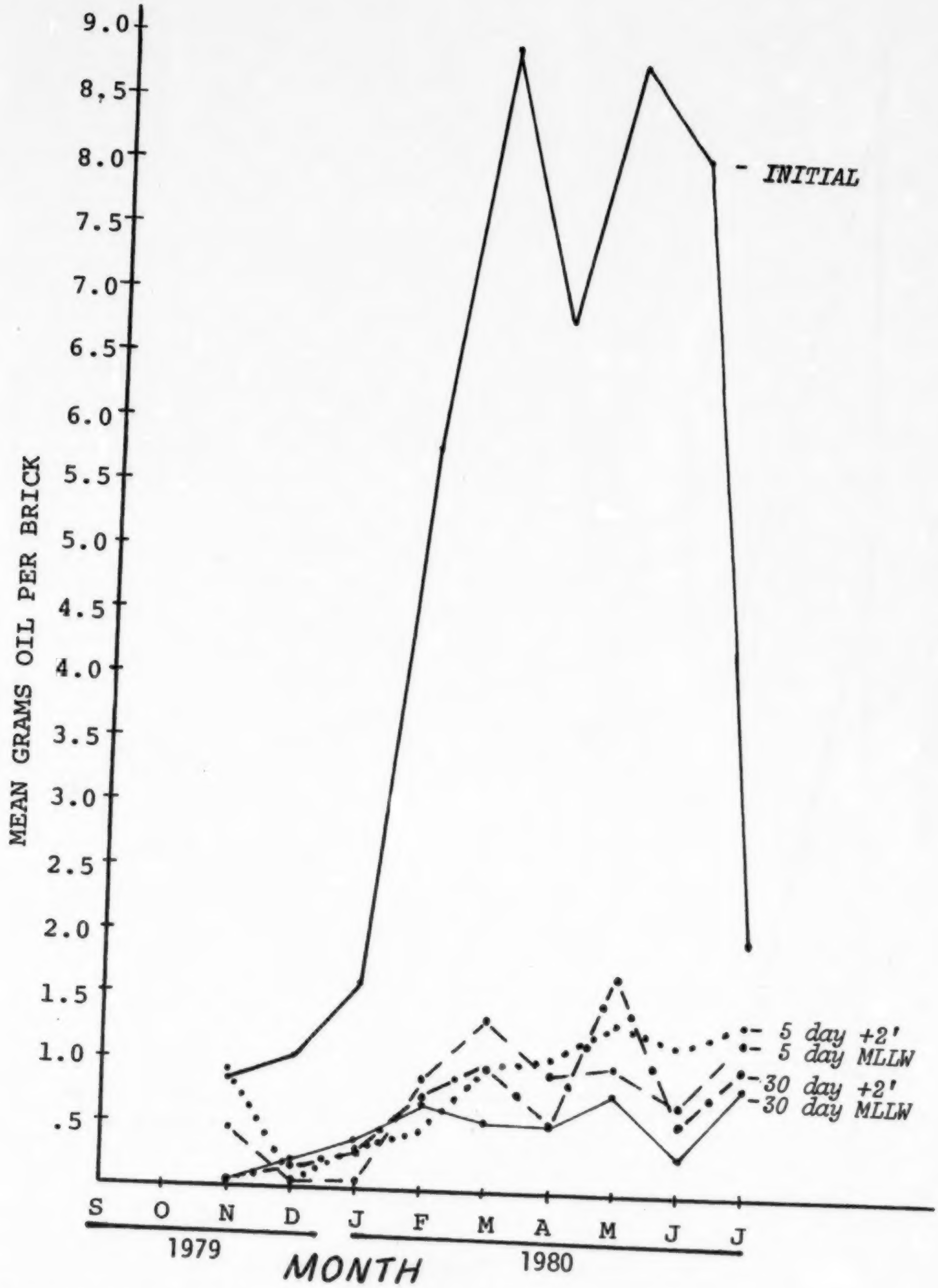

Figure 20. Monthly mean concentrations of total oil on top surface of experimental substrates (N = 5 measurements per point) at Sequim Bay.

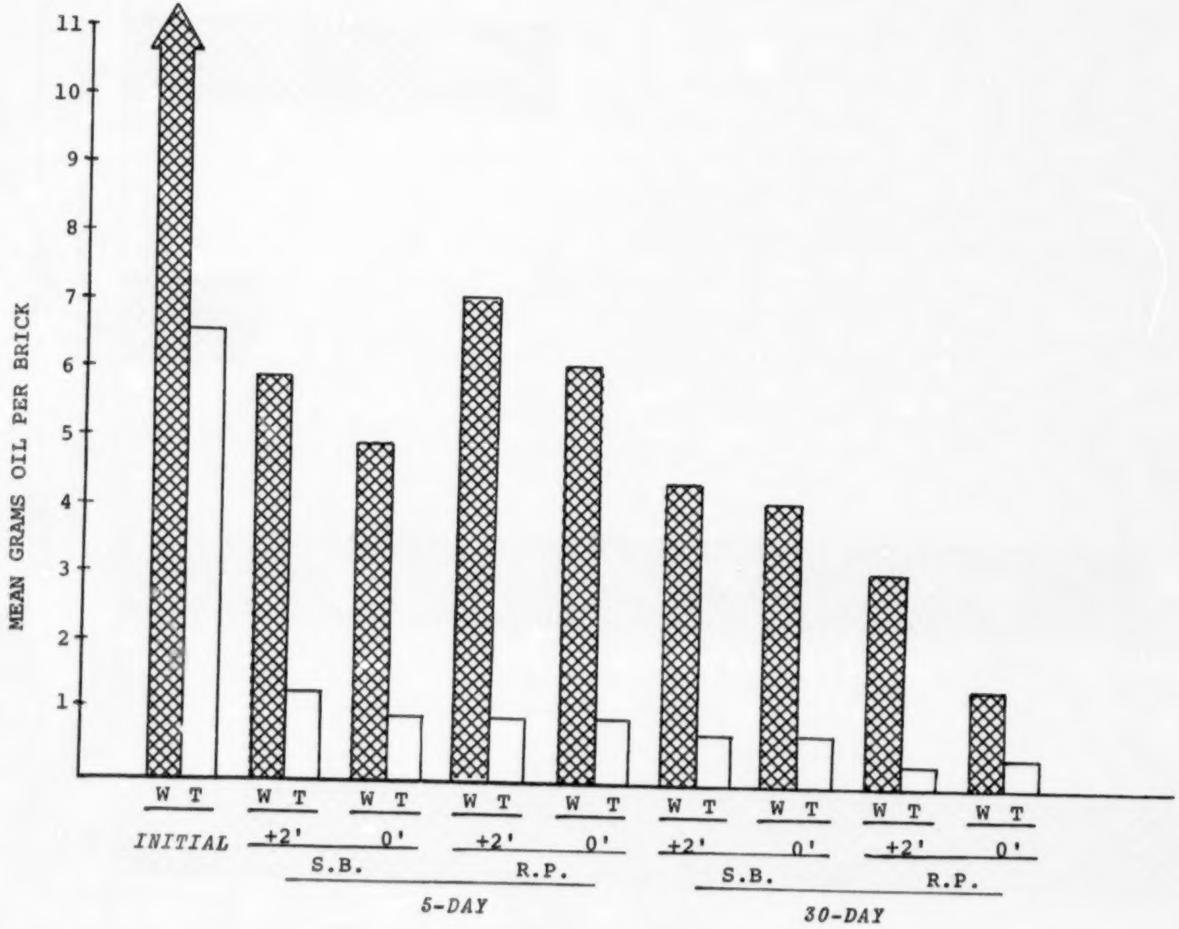

Figure 21. Total oil concentration in hard substrate site experiments by site, tide level, and field exposure time (R.P. = Rocky Point; S.B. = Sequim Bay; W - whole brick extraction; T = top surface extraction; 0' = MLLW; and +2' = 2' above MLLW).

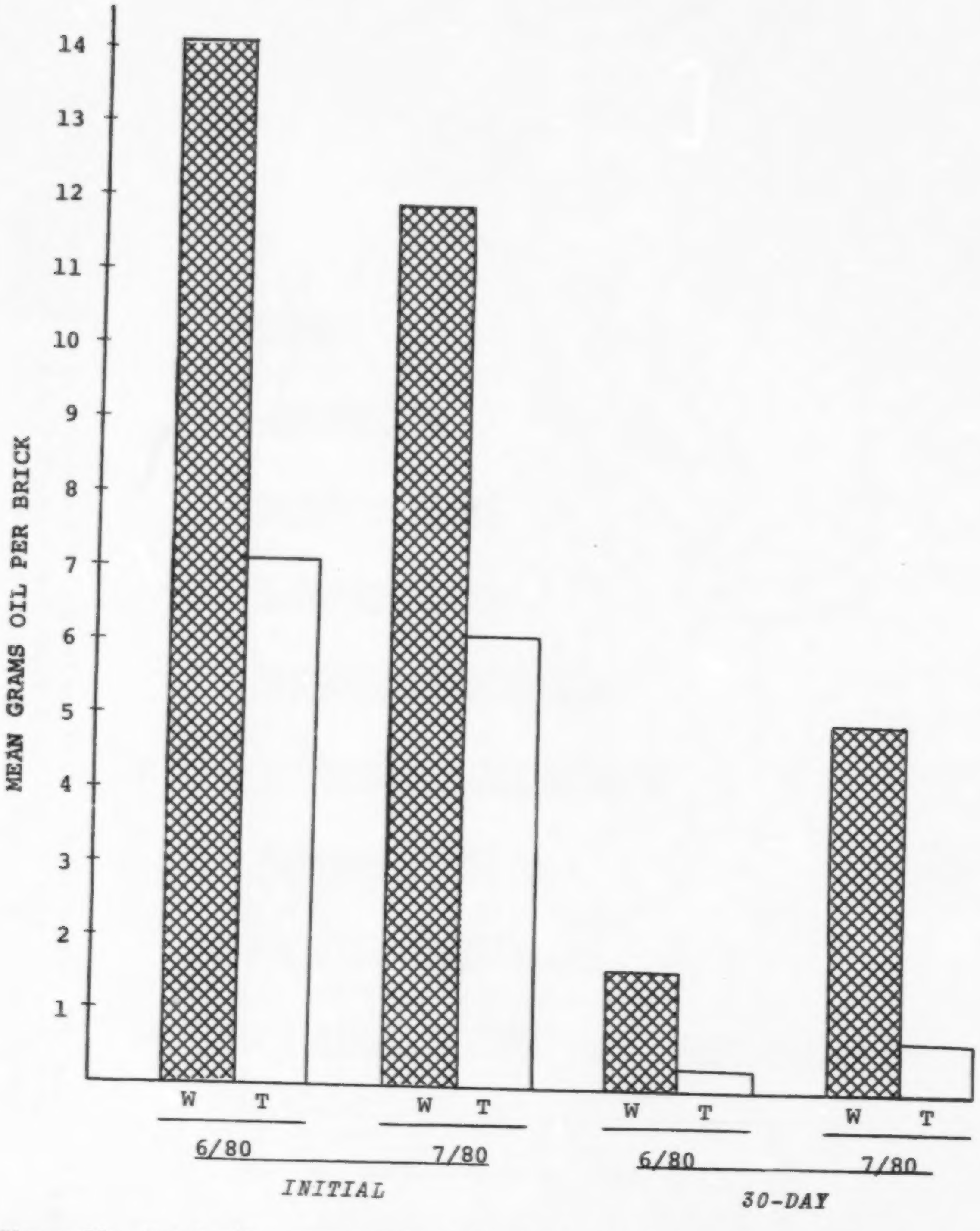

Figure 22. Total oil concentration in hard substrate site experiments at MLLW by month of experiment (W = whole brick extraction; T = top surface extraction). Data summarized over two sites (Sequim Bay and Rocky Point).

July) is in Figure 22. These data show a slightly higher initial concentration of total oil for whole brick extractions and top surface extractions in June as compared to July. For the 30-day MLLW data, the opposite is true. The data merely reflect the normal variation associated with the methods, which was more clearly shown in Table 11.

Hard Substrate Recovery - Analyzed Saturates and Aromatics

Monthly Experiments. Samples analyzed by capillary gas chromatography identified the compounds listed on Table 12. Example concentrations of individual compounds from samples taken immediately post-treatment and at five and 30 days after field placement in the April 1980 experiment are also shown in Table 12. These data and similar data from four other sets of replicates sampled during the April experiment provide the basis for the summed saturate and aromatic compound concentrations given in Figures 23-26. The total saturate compound data is based on a sum of the 19 saturate compounds analyzed, and the total aromatic compound data is based on a sum of the 12 aromatic compounds analyzed. The sum of these classes does not and should not add to comprise total oil, as measured by infrared spectrophotometry since it includes only a few of the many components in oil.

Data on the time course of analyzed saturate compounds in the April 1980 experiment are shown in Figure 23. The data for MLLW follow a time course which parallels the data on total oil for the experiments overall (Figure 19). One should note that total oil concentrations are in grams of oil per brick while total saturate and aromatic compounds are indicated in milligrams of oil per brick. The data for the +2' above MLLW tide level show a much greater retention of total analyzed saturate compounds during the first five days of field exposure than for MLLW and the opposite at 30 days.

The time course of analyzed aromatic compounds during the April 1980 experiment is shown in Figure 24. The loss of these compounds from experimental substrates at the +2' tide level and MLLW was about equal at five days. A greater loss was indicated for the +2' tide level at the end of 30 days. The sample sizes for computing these means were much smaller (N = 5 bricks per mean) than for the total oil data (N = 50 bricks per mean), and the indicated discrepancy is within the methodological sensitivity.

Comparative data for the whole brick and top surface extractions at the 30-day time interval are in Figure 25. The top surface extractions are directionally compatible with whole brick extractions and also show a greater concentration of both analyzed saturate and aromatic compounds at MLLW as compared to +2' above MLLW. For saturate compounds, the top surface extractions represented about 37% of whole brick extractions at both MLLW and at +2' above MLLW. For aromatic compounds, the top surface extractions amounted to nearly 50% of whole brick extractions. These proportions exceed somewhat the 20% top surface/whole brick relationship for total oil indicated in Table 11.

Table 12. List of saturate and aromatic compounds identified by gas capillary chromatography in hard substrate recovery experiments.*

COMPOUND	EXAMPLE WHOLE BRICK CONCENTRATIONS (mg/brick)		
	INITIAL	5-DAY	30-DAY
SATURATES			
C_{12}	8.80	4.33	0.10
C_{13}	13.67	7.83	0.18
C_{15}	28.05	14.64	0.31
C_{16}	27.45	18.15	0.31
C_{17}	29.69	18.26	0.33
PRISTANE	19.57	11.88	0.40
C_{18}	26.17	15.50	0.31
PHYTANE	12.05	7.52	0.34
C_{19}	27.29	15.73	0.19
C_{20}	23.59	13.46	0.38
C_{21}	21.06	11.98	0.30
C_{22}	18.81	11.74	0.29
C_{23}	16.37	10.92	0.26
C_{24}	15.01	9.95	0.23
C_{25}	12.21	8.29	0.21
C_{26}	10.51	7.25	0.17
C_{27}	6.18	4.06	0.15
C_{28}	4.00	2.45	0.08
			0.11
AROMATICS			
NAPHTHALENES			
NAPTHALENE	0.12	0.008	0.001
2 MN	4.38	0.755	0.032
1 MN	3.63	0.167	0.032
1E, 2E	0.95	0.27	0.013
2,6 2,7	4.98	0.67	0.053
1,3 1,6	4.27	0.77	0.017
1,7	4.66	0.96	0.047
1,4 2,3 1,5	2.88	0.73	0.025
1,2	1.44	0.26	0.011
PHENANTHARENES			
PHENANTHARENE	0.95	0.38	0.011
C_1	0.35	0.13	0.006
C_2	1.04	0.28	0.015

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* These example data represent a single replicate from the April 1980 experiment at Sequim Bay, MLLW.

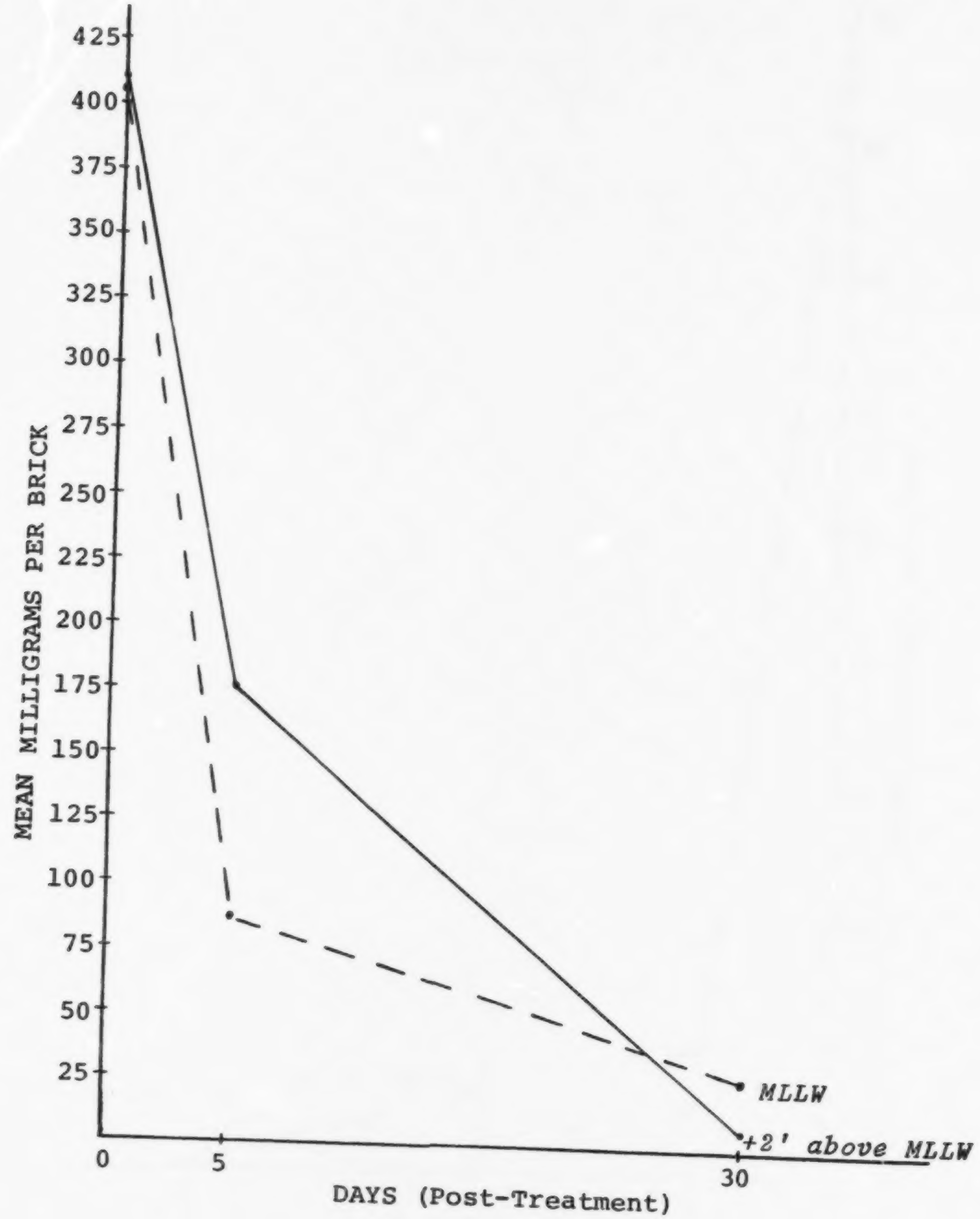

Figure 23. Summary of measured saturate compounds in terms of individual experiment time frames (N = 5 measurements per point) in monthly experiments at Sequim Bay.

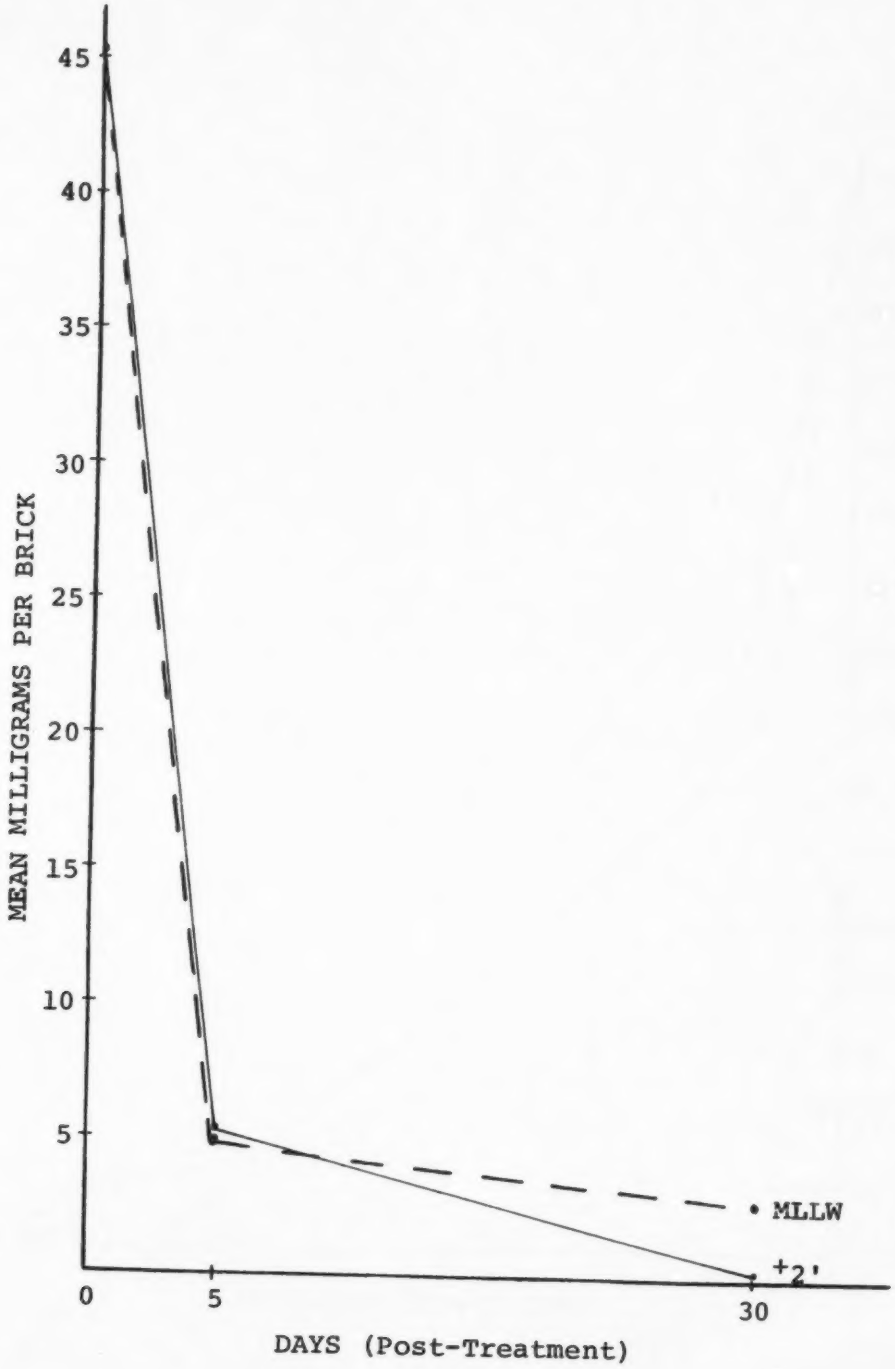

Figure 24. Summary of measured aromatic compounds in terms of individual experiment time frames (N = 5 measurements per point) in monthly experiments at Sequim Bay.

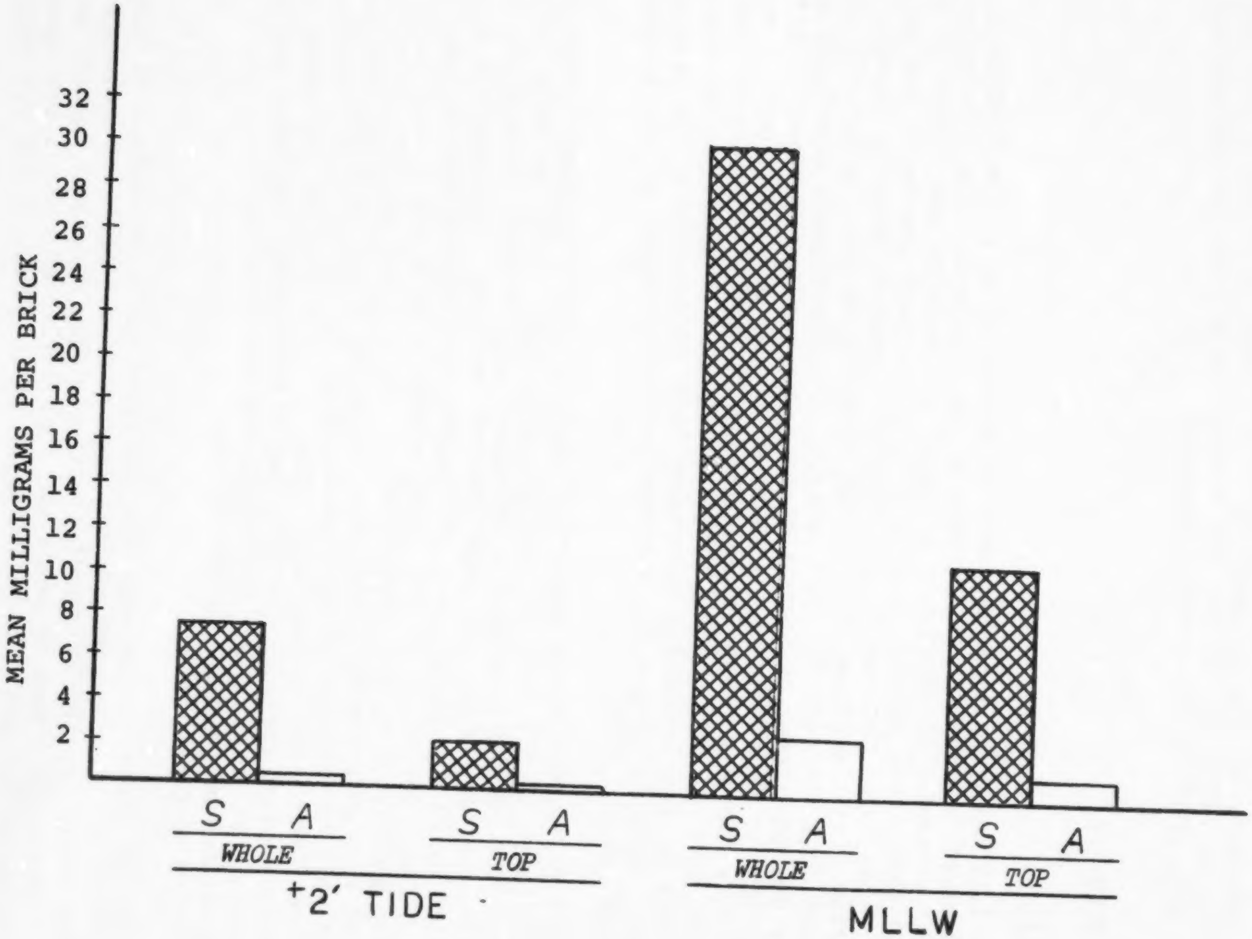

Figure 25. Comparison of tide levels in terms of measured saturate and aromatic compounds (N = 5 measurements per bar) 30 days after treatment at Sequim Bay (S = analyzed saturates; A = analyzed aromatics).

The data on Figure 26 represent a preliminary attempt to partition oil associated with bricks at MLLW, 30-day field exposure, during the April experiment between organisms on bricks and the bricks themselves. The comparison in analyzed saturate and aromatic compounds is between bricks which were scraped free of organisms and those with normal 30-day colonization. In terms of whole brick extractions, the difference between organism-free bricks and colonized bricks is proportionally quite small for saturate compounds but may be appreciable for aromatics. The proportions attributable to organisms in the top surface extractions is apparent and large for both saturate and aromatic compounds.

CLAM BED RECOVERY

A Perspective

This task was principally designed to measure effects from Prudhoe Bay crude oil mixed in sediments on recovery by the littleneck clam (Protothaca staminea), a commercial species. In terms of longevity and frequency of successful sets, this species is more akin to dominant species in the rock habitat. Thus, the three-month experimental framework directly addresses the question of sediment suitability for reseeding by the clam but not the longer term full recovery for this species. The three-month experiment at Discovery Bay duplicated an experiment conducted during the summer of 1979 at Sequim Bay for MLLW with the exception of initial oil concentration, and included data for nine other primary species and the entire infaunal community, as well as data on the littleneck clam. A comparison of these summary data to the Sequim Bay experiment lends perspective to the present experiment. Data for Sequim Bay are from an interim report on this project (Vanderhorst et al., 1980).

Figure 27 represents the numbers of species within taxonomic groups in this experiment (D.B.) and the earlier experiment (S.B) at MLLW. The numbers of species represented by the equal sample sizes in controls is remarkably similar, with the exception of mollusks. Polychaetes were represented by 21 and 20 species; crustaceans by 13 and 14 species; and the "other" group, consisting of all other species, by 6 and 7 species for Sequim Bay and Discovery Bay, respectively. The mollusks were an exception since they were only a minor constituent at Sequim Bay (3 species) and were nearly as well represented as the crustaceans at Discovery Bay (12 species). There were fewer species for each category in the oiled sediments as compared to unoiled controls for this task's experiment. Mollusks were an exception to that trend in the previously reported experiment. Polychaetes were represented by the most species followed by crustaceans, mollusks, and all remaining species.

A similar comparison between the two experiments for numbers of individuals is in Figure 28. The natural logarithm of numbers of individuals per square meter is used to permit plotting widely divergent numbers on a single figure. The range in means was from 3 (21 individuals per square meter (oiled, other species, Sequim Bay)) to 11 (about 50,000 per square

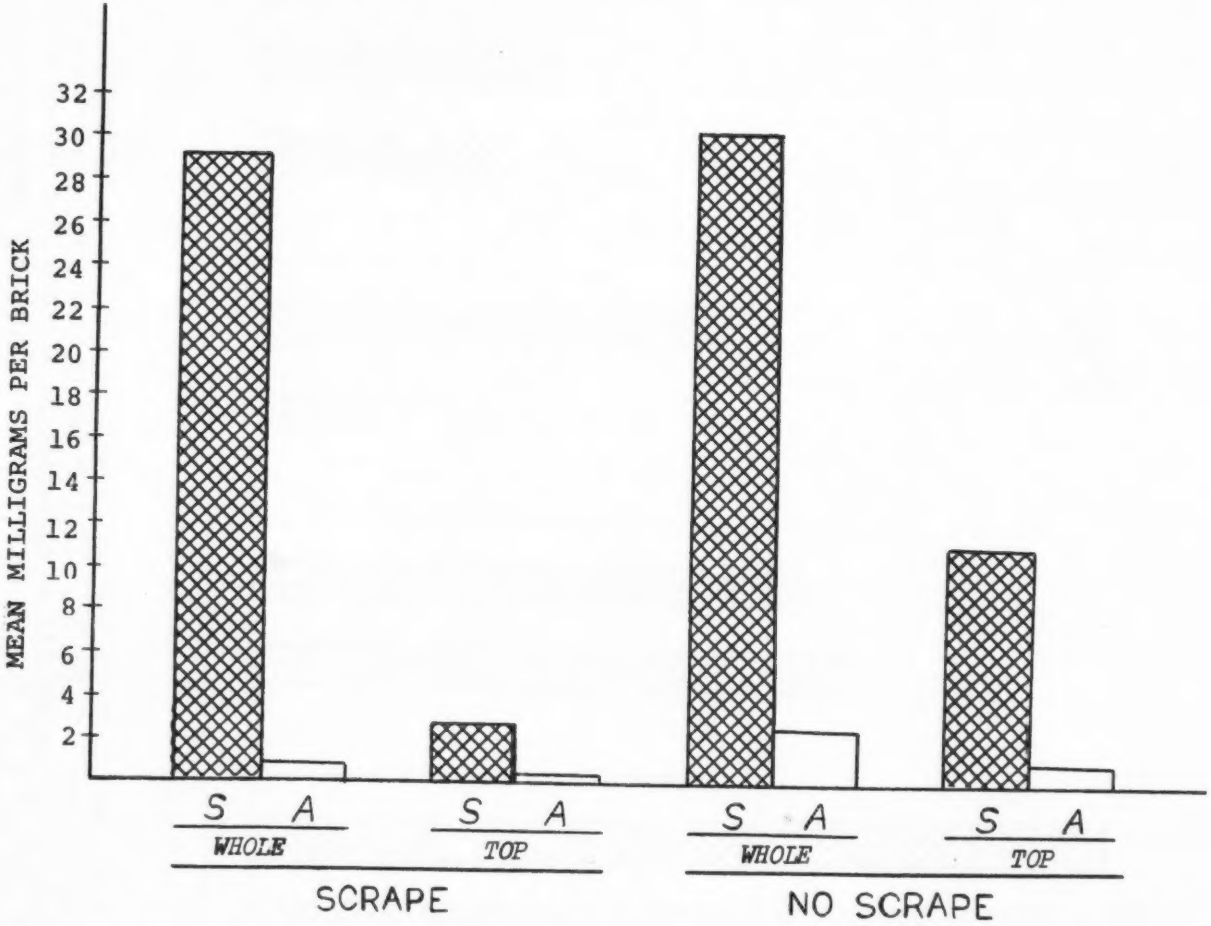

Figure 26. Comparisons of scraped and unscraped bricks in terms of measured saturate and aromatic compounds at MLLW tide level at Sequim Bay (N = 5 measurements per bar) 30 days after oil treatment. Scraped bricks were organism-free when analyzed; unscraped bricks had that complement of organisms colonized in 30 days (S = analyzed saturates; A = analyzed aromatics).

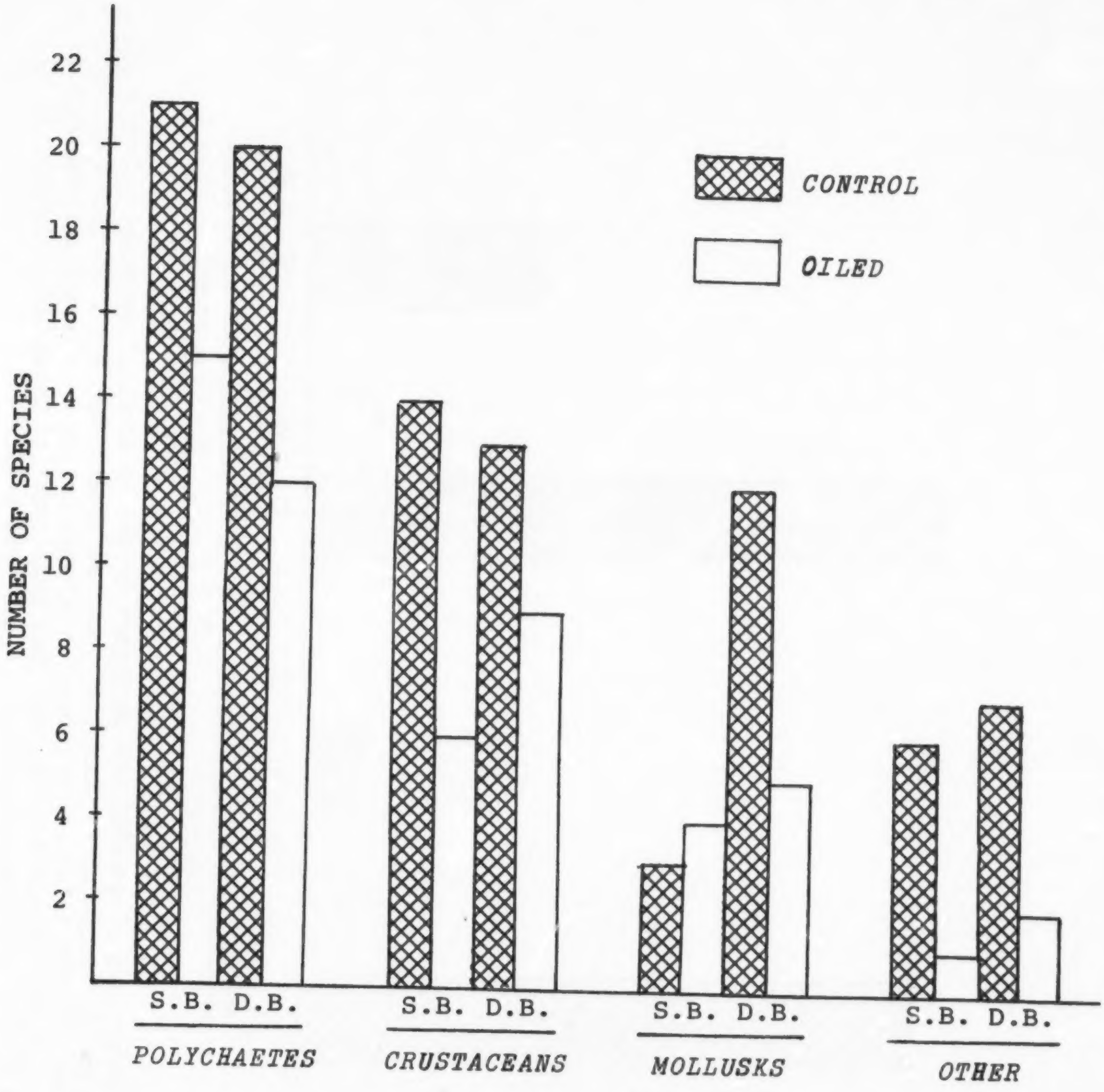

Figure 27. Comparison of Sequim Bay and commercial clam bed at Discovery Bay in terms of species. (S.B. = Sequim Bay; D.B. = Discovery Bay; number of species is aggregate in 35 cores distributed in 5 replicates of 7 cores each for each condition.) Samples were collected 3 months after oil treatment during the spring-summer season.

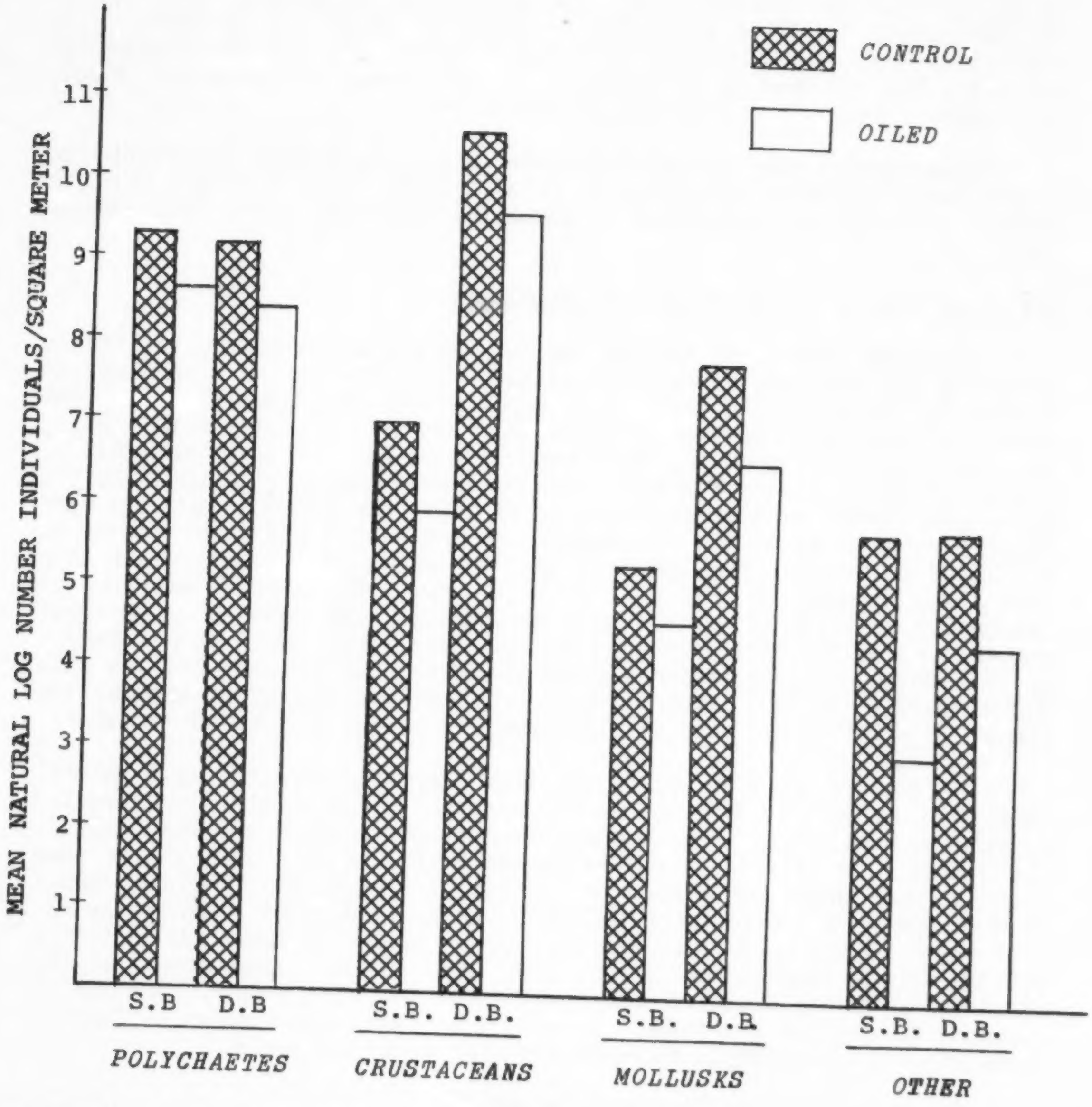

Figure 28. Comparison of Sequim Bay and commercial clam bed at Discovery Bay in terms of natural log of numbers of individuals/square meter. (S.B. = Sequim Bay; D.B. = Discovery Bay.) Samples were collected 3 months after oil treatment in the spring-summer season. Each mean is based on the natural logarithm of numbers of individuals in 35 cores distributed in 5 replicates of 5 cores each for each condition.

meter (control, crustaceans, Discovery Bay)). Numbers of polychaetes were about the same at the two sites as were numbers of individuals in the "other" species group. There were more individual crustaceans and individual mollusks at Discovery Bay. In every case there were fewer numbers of individuals in oiled sediments as compared to unoiled controls. The highest number of individuals per square meter was for crustaceans at Discovery Bay followed by polychaetes at both sites, mollusks in controls at Discovery Bay, and crustaceans at Sequim Bay.

These summary data indicate that the present experiment replicated the Sequim Bay experiment (Vanderhorst et al., 1980) very well and that the overall recovery was proceeding at about the same rate as for that experiment.

Effect of Tide Level on the General Community

Since the number of species represented at MLLW was very similar at Discovery Bay and Sequim Bay, we have used data from the -2' tide level at Sequim Bay to help illustrate tide level trends in community data. The number of species from each of three tide levels (-2', Sequim Bay; MLLW, Discovery Bay; +2', Discovery Bay) are in Figure 29. For unoiled controls, the polychaetes, the crustaceans, and the "other" species group exhibit a decreasing number of species from low to high tide levels. Mollusks were equally represented at the two upper tide levels, and had fewer species at the -2' tide level. This may very well be an effect of site rather than tide level because of the generally low representation of mollusks at Sequim Bay. At the +2' tide level, polychaetes were represented by equal numbers of species in treated and control sediments. The oiled sediments at this tide level contained a greater number of crustacean species than did controls. At all other tide levels and for the other groups, the number of species in controls exceeded the number in oiled sediments.

The numbers of individuals per square meter (expressed as a natural logarithm) as related to tide level are shown in Figure 30. The trend for polychaetes and "other" species was from a higher number of individuals at the lowest tide to a lower number at the highest tide. This is the same trend as was shown for number of species in Figure 29. Mollusks had a greater number of individuals per square meter at MLLW followed by the +2' tide level. Crustaceans also had the highest number of individuals at MLLW but had higher numbers at the -2' level than at +2'. Crustaceans at the +2' tide level had slightly more individuals per square meter in oiled as compared to control sediments. In all other cases the numbers of individuals in control sediments exceeded that for oiled sediments.

Analysis of Variance for Taxonomic Groups

The data on numbers of individuals and species in the commercial clam bed experiment are presented in a different way in Table 13. The mean numbers of individuals and species per tray are tabulated in terms of oil treatment and tide level. Analyses of variance were computed on the data resulting in these means to distinguish statistically significant (P =

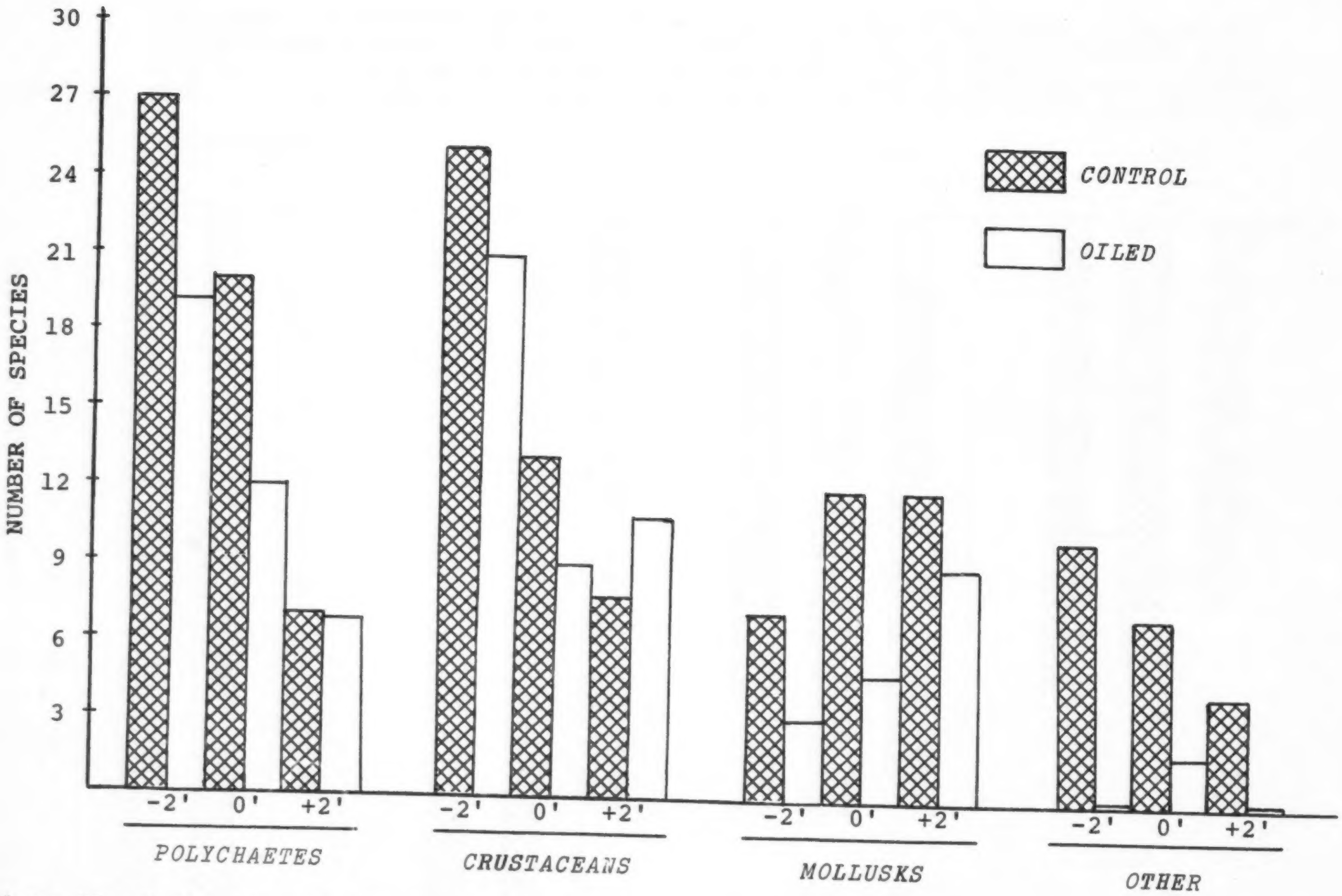

Figure 29. Number of species within taxonomic groups in commercial clam bed experiment distributed by tide level. Number of species is aggregate of 35 cores distributed in 5 replicates of 7 cores each per condition. (-2' = 2' below MLLW at Sequim Bay, 1980.) Both experiments were for 3 months after oil treatment in the spring-summer season.

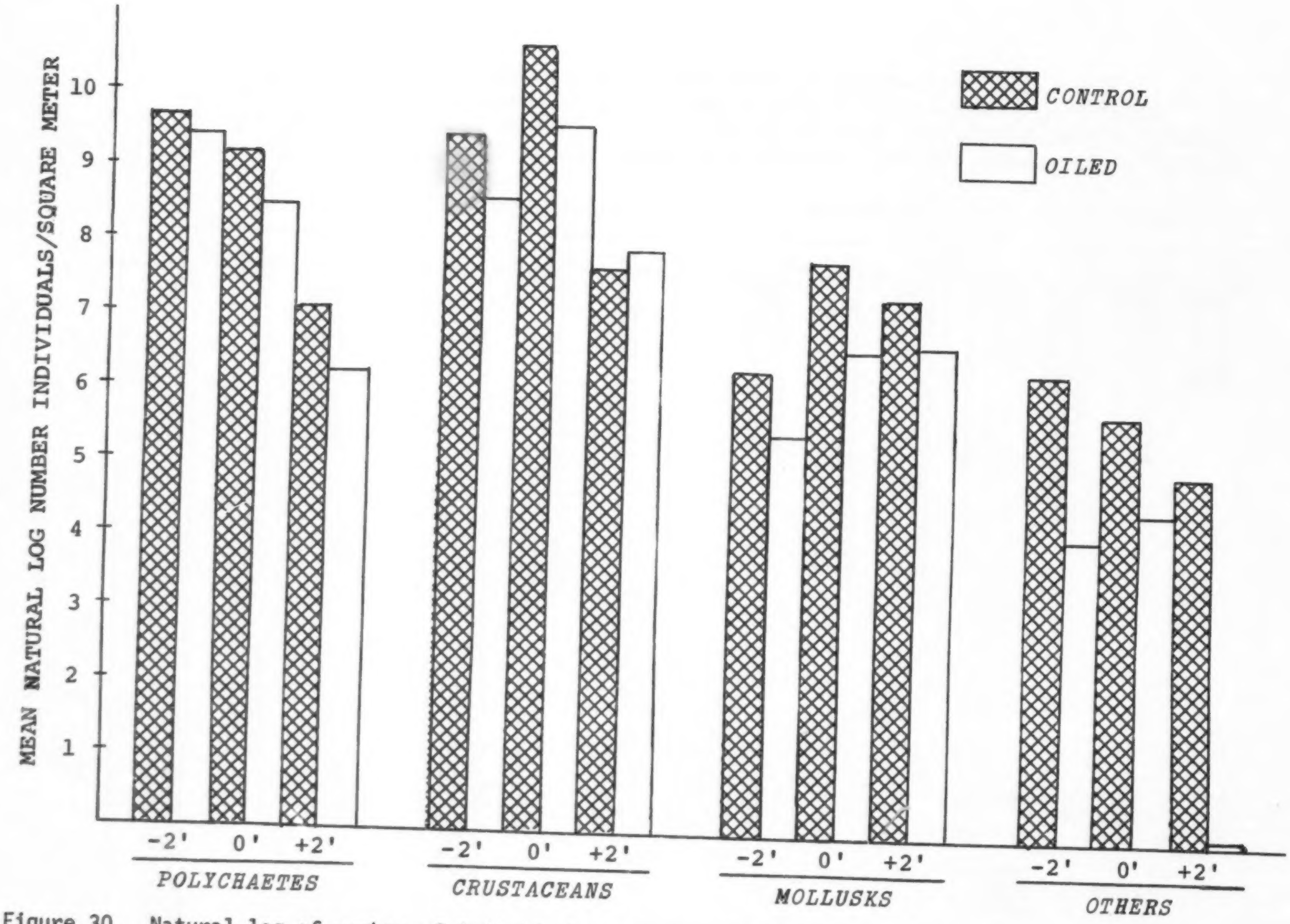

Figure 30. Natural log of number of individuals/square meter within taxonomic groups distributed by tide level. (-2' = 2' below MLLW data from Sequim Bay, 1979; 0' MLLW and +2' = 2' above MLLW data from Discovery Bay, 1980.) Each mean is based on 35 cores distributed in 5 replicates of 5 cores per replicate and represents 3 months colonization in the spring-summer season.

Table 13. Summary of mean numbers of individuals and species in commercial clam bed recovery experiment at Discovery Bay for a 3-month spring-summer period in 1980.

	NUMBERS/TRAY									
	OIL EFFECTS				TIDE LEVEL EFFECTS				STATISTICAL SIGNIFICANCE[1]	
	CONTROLS		OILED		MLLW		+2' ABOVE MLLW			
	Mean	(S.D.)	Mean	(S.D.)	Mean	(S.D.)	Mean	(S.D.)	Oil	Tide
INDIVIDUAL NUMBERS										
POLYCHAETES	7.65	(0.43)	3.71	(0.43)	10.86	(0.43)	1.29	(0.43)	Yes	Yes
CRUSTACEANS	31.91	(2.82)	12.31	(2.82)	40.56	(2.82)	3.49	(2.82)	Yes	Yes
MOLLUSKS	2.91	(0.24)	1.05	(0.25)	2.20	(0.25)	1.77	(0.25)	Yes	No
SPECIES NUMBERS										
POLYCHAETES	2.92	(0.14)	1.31	(0.14)	3.36	(0.14)	0.88	(0.14)	Yes	Yes
CRUSTACEANS	2.06	(0.23)	1.68	(0.24)	1.78	(0.24)	1.96	(0.24)	No	No
MOLLUSKS	1.87	(0.15)	0.84	(0.15)	1.34	(0.15)	1.37	(0.15)	Yes	No
TOTAL	7.15	(0.38)	3.90	(0.38)	6.76	(0.38)	4.30	(0.38)	Yes	Yes

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[1] Deemed statistically significant with alpha probability less than or equal to 0.05. Based on 35 cores distributed in 5 replicates of 5 trays each per treatment.

0.05) effects due to oil and tide level. There were significantly more individual polychaetes, crustaceans, and mollusks per tray in control sediments as compared to oiled. There were significantly more individual polychaetes and crustaceans at the MLLW tide level as compared to the +2' tide level. A slightly higher (0.43 individuals per tray) mean number of individual mollusks at MLLW as compared to +2' tide level was not statistically significant.

Total number of species per tray and numbers of polychaete species per tray were significantly higher in control substrates as compared to oiled, and at MLLW tide level as compared to +2'. Numbers of species of mollusks per tray were significantly higher in control substrates as compared to oiled. A slightly higher (0.03 per tray) mean number of species per tray at the +2' tide level as compared to the MLLW tide level for mollusks was not statistically significant.

Taxonomic and Trophic Composition

A total of 70 species were sampled in the commercial clam bed experiment (Table 14). Crustacean species were best represented with 25 species (36%); followed by polychaetes with 23 species (33%); mollusks, 16 species (23%); and all other species, six species (10%). This can be compared to MLLW controls at Sequim Bay (Vanderhorst et al., 1980) where polychaetes represented 48%; crustaceans, 32%; mollusks, 7%; and all other species, 14%. The basic difference in composition relates to the greater number of mollusk species at Discovery Bay.

The overall trophic composition (Table 14) derived from Simenstad et al. (1979) shows a dominance of detritivores, 23 species (33%); followed by carnivores, 17 species (24%); herbivores, 14 species (20%); and suspension-feeders, nine species (13%). Eight species (11%) had either reportedly varied trophic classification, or did not fit into our chosen four basic groups.

The trophic classification differed between taxonomic categories. Polychaetes were dominated by detritivores, 12 species (52%); and carnivores, seven species (30%). There was one herbivore among the polychaetes and no suspension-feeders. Three species had varied or other trophic classifications. The crustaceans also had a predominance of detritivores, nine species (36%), but had the most even trophic distribution of all the taxonomic groups: carnivores, four species (16%); suspension-feeders, five species (20%); herbivores, six species (24%); and varied or other, one species (4%). Mollusks had more herbivores, seven species (44%), than other trophic groups. This amounted to 50% of all herbivore species. The other trophic groups within the mollusks were: suspension-feeders, four species (25%); detritivores, two species (13%); and a single carnivore species (6%). Two species did not fall in the four basic trophic categories we had adopted.

The composition of trophic groups expressed as numbers of species within the tide level and oil treatments is shown in Figure 31.

Table 14. Species composition and trophic groups for commercial clam bed recovery experiment.[1]

TAXONOMIC GROUPS/SPECIES	TROPHIC GROUPS[2]
POLYCHAETES	
Armandia brevis	detritivore
Axiothella rubrocincta	detritivore
Boccardia proboscidea	-
Capitella capitata	detritivore
Capitellid undet.	detritivore
Cirratulid undet.	detritivore
Exogone lourei	detritivore
Glycinde armigera	carnivore
Goniadid undet.	carnivore
Halosynda brevisetosa	carnivore
Hemipodus borealis	carnivore
Maldanid undet.	-
Nothria elegans	
Notomastus (Clistomastus) tenuis	detritivore
Ophiodromus pugettensis	canivore
Owenia fusiformis (= collaris)	detritivore
Phyllodoce (Anaitides) maculata	carnivore
Platynereis bicanaliculata	herbivore
Polychaete undet.	varied
Polydora socialis	detritivore
Protodorvillea gracilis	carnivore
Spio filicornis	detritivore
Spionid undet.	detritivore
CRUSTACEANS	
Allorchestes angusta	detritivore
Ampelisca pugetica	detritivore
Amphipod undet.	various
Anisogammarus confervicolus	herbivore
Aoroides columbiae	detritivore
Balanus sp.	suspension
Caprella sp.	herbivore
Corophium ascherusicum	detritivore
Cumella vulgaris	detritivore
Eualus townsendi	carnivore
Exosphaeroma amplicauda	herbivorus scavenger
Exosphaeroma sp.	herbivorus scavenger
Gammaropsis	suspension

Table 14. (Continued)

TAXONOMIC GROUPS/SPECIES	TROPHIC GROUPS[2]
CRUSTACEANS (Continued)	
Gnorimosphaeroma o. oregonensis	herbivorous scavenger
Hemigrapsus nudus	carnivore
Heptacarpus paludicola	carnivore
Heptacarpus sp.	carnivore
Leptochelia dubia	detritivore
Nebalia pugettensis	suspension
Parallorchestes ochotensis	detritivore
Paraphoxus sp.	detritivore
Photis brevipes	suspension
Photis sp.	suspension
Pugettia gracilis	herbivore
Upogebia pugettensis	detritivore/suspension
MOLLUSKS	
Acmaea sp.	herbivore
Alvania compacta	herbivore
Caecum occidentale	-
Lacuna variegata	herbivore
Littorina scutulata	herbivore
Littorina sitkana	herbivore
Macoma inquinata	detritivore
Macoma sp.	detritivore
Margarites pupillus	herbivore
Mysella tumida	suspension
Mytilus edulis	suspension
Nassarius mendicus	carnivore
Notoacmea persona	herbivore
Protothaca staminea	suspension
Solariella sp.	-
Transennella tantilla	suspension
OTHER SPECIES	
Amphipholis sp.	-
Leptosynapta clarki	-
Nemertea undet. (sp. A)	carnivore
Nemertea undet. (sp. B)	carnivore
Paranemertes peregrina	carnivore
Sipunculid undet.	carnivore

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[1] Experiment at Discovery Bay, MLLW and +2' above MLLW with 3-month colonization during spring and summer, 1980.

[2] Trophic classification from Simenstad et al. (1979).

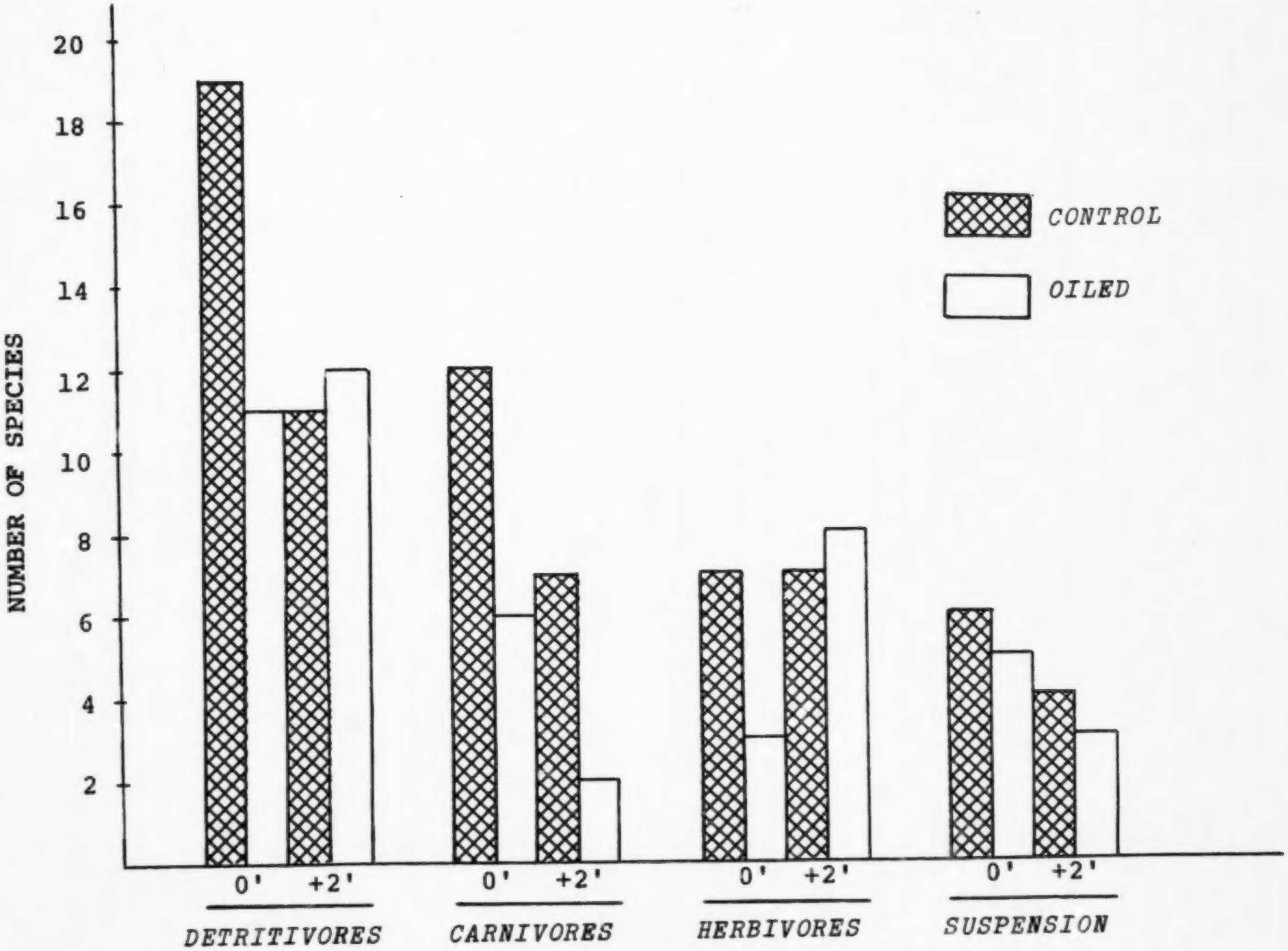

Figure 31. Number of species within trophic groups in commercial clam bed recovery experiment. Number of species is aggregate in 35 cores distributed in 5 replicates per condition. (0 = MLLW; +2' = 2' above MLLW.) Experiment was at Discovery Bay for 3 months during the spring-summer season, 1980.

Detritivores were much better represented at MLLW, in controls, than at +2'. There were also eight fewer species in oiled sediments as compared to controls at MLLW. The number of species of detritivores in oiled sediments at +2' exceeded that for controls by one species. Carnivores were also much better represented at MLLW than at +2'. There were fewer species in oiled sediments at each of the tide levels. Numbers of species of herbivores in controls were equal (seven species) at each of the tide levels. There were fewer (four species) in oiled sediments at MLLW and one more species in oiled sediments than controls at +2'. Suspension-feeders were represented by a greater number (total six species) of species at MLLW and in control sediments at both tide levels.

The density of trophic groups related to oil treatment and tide level, expressed as the natural logarithm of numbers of individuals per square meter, is shown in Figure 32. Detritivores had higher density at MLLW than at +2' tide level and higher density in control sediments compared to the respective oiled sediments. The same trend was true for carnivores and suspension-feeders although for carnivores the tide level difference between controls was minimal. Herbivore densities were roughly equivalent in all of the tide level and oil treatment categories.

Primary Species

Tray densities for the ten primary species which were *a priori* selected for testing of oil effects hypotheses are shown in Table 15. With the exception of *Lacuna* sp. (MLLW, +2' tide levels), *Corophium ascherusicum* (+2' tide level), and *Platynereis bicanaliculata* (MLLW), all mean densities were equal to or higher in controls than in oiled sediments.

In control sediments, there were higher densities at MLLW than at +2' for all species except the littleneck clam (*Protothaca staminea*). In oiled sediments there were higher densities at the +2' tide level for *Protothaca staminea*, *Corophium ascherusicum*, and *Leptochelia dubia*. *Photis brevipes* never occurred in oiled sediments.

In control sediments, *L. dubia* far exceeded all other species in density. *Exogone lourei* was the second highest species in density at MLLW. These two species were identified as particularly good subjects for the experimental indication of oil treatment effects on recovery of infauna in our region based on Sequim Bay and Protection Island data (Vanderhorst et al., 1980). Their high density and the differential in density between oiled and unoiled sediments tends to confirm their value for such use.

The overall density for primary species was much higher (498 individuals per tray) for this experiment as compared to the equivalent experiment at Sequim Bay in 1979 (54.4 individuals per tray). Most of this difference is attributable to the density of *Leptochelia dubia* although there was higher density in MLLW controls for each of the primary species except *Photis brevipes* and *Polydora socialis*.

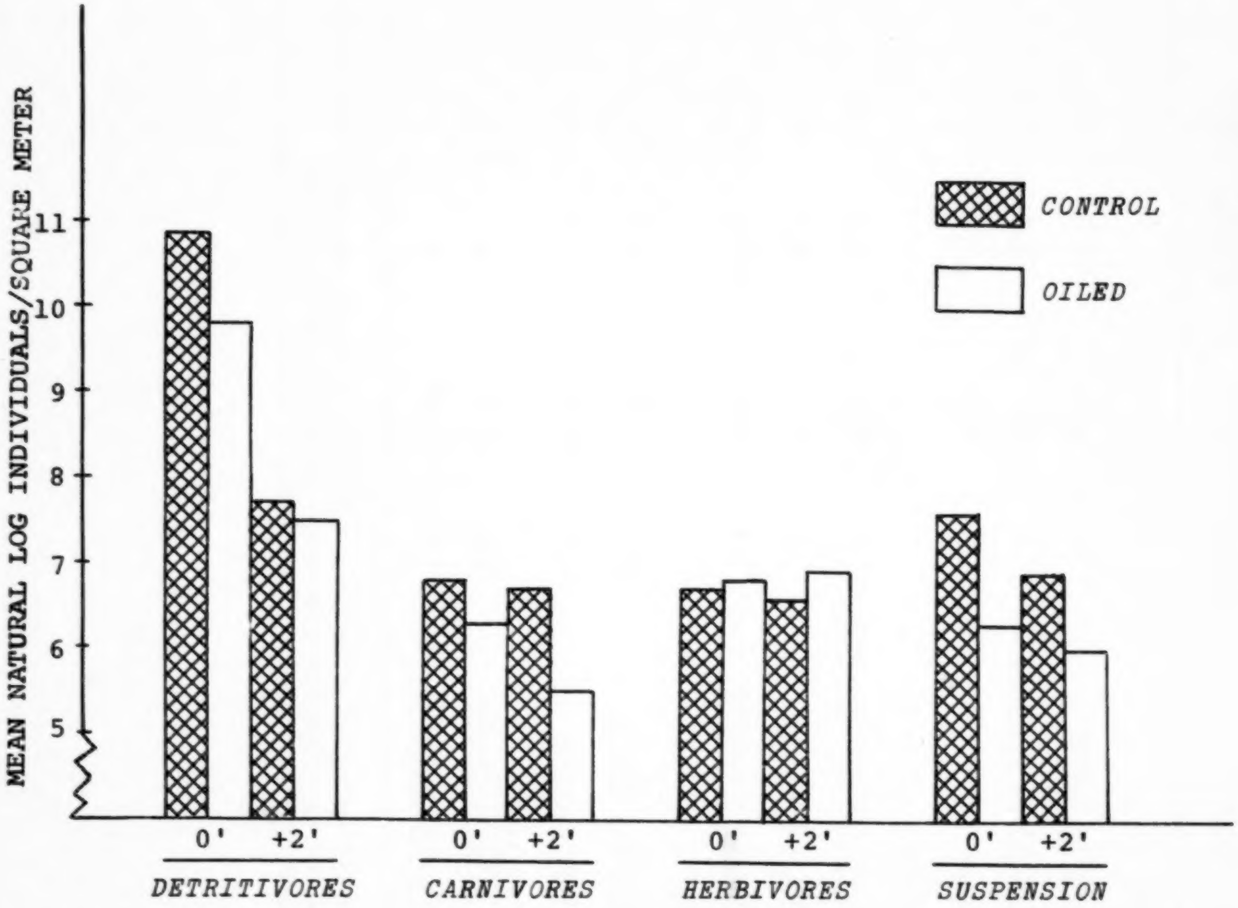

Figure 32. Natural log of number of individuals/square meter with trophic groups in commercial clam bed recovery experiment. (0 = MLLW; +2' = 2' above MLLW.) Each mean is based on 35 cores distributed in 5 replicates per condition. Experiment was at Discovery Bay for 3 months during the spring-summer season, 1980.

Table 15. The mean density of primary species in commercial clam bed experiment (tide level, oil, three-month recovery summary).

PRIMARY SPECIES	MEAN NUMBERS/TRAY MLLW Control	 Oil	 +2' Above MLLW Control	 Oil
MOLLUSKS				
Mysella tumida	12.9	4.0	4.0	1.2
Protothaca staminea	2.9	0.1	3.3	2.6
Lacuna sp.	1.8	2.2	0.2	0.2
CRUSTACEANS				
Corophium ascherusicum	11.2	0.4	0.0	1.4
Photis brevipes	0.3	0	0.2	0
Leptochelia dubia	405.3	6.8	137.8	11.2
POLYCHAETES				
Armandia brevis	10.5	3.5	0.0	0.0
Exogone lourei	47.6	19.4	0.6	0.3
Platynereis bicanaliculata	4.2	6.4	0	0
Polydora socialis	1.7	0.6	0	0

Means based on 5 replicates per condition (7 cores per replicate tray). Analyses of variance for tide and treatment effects on Table 16. Experiment was at Discovery Bay during a 3-month spring-summer period, 1980.

Hypothesis tests for the main effects attributable to oil and tide level are shown in Table 16. Statistically significant effects due to tide level were demonstrated for seven of the ten species. The exceptions were *Protothaca staminea*, *Corophium ascherusicum*, and *Photis brevipes*. This indicates a much larger tide level effect in this experiment where the comparison was between MLLW and +2' as compared to the equivalent experiment at Sequim Bay (Vanderhorst et al., 1980) where the comparison was between MLLW and -2'. In that experiment significant tide level effects were demonstrated for only four of ten species.

Significant effects due to oil treatment were demonstrated for the densities of four of the ten primary species: *Mysella tumida*, *Protothaca staminea*, *Leptochelia dubia*, and *Armandia brevis*. For comparison, there were also four of the 10 species having significant effects due to oiling in the equivalent Sequim Bay experiment (Vanderhorst et al., 1980).

Species With Indicated Oil Treatment Effects

For reasons stated in the methods and in Vanderhorst et al.(1980), we used *a priori* selected species for hypothesis tests to ensure a conservative estimate of oil treatment effects. However, analyses of variance of the density for all 70 species in this experiment were computed. Statistically significant (P = 0.05) effects on density attributable to the oil treatment were indicated for nearly a third (21) of the 70 species (30%). These species, with attendant trophic designation are listed on Table 17.

It is of interest to compare the contribution of species to trophic and taxonomic groups as related to oil treatment. We have done this in index form as follows. For the denominator of the index we use the percentage contribution based on numbers of species in taxonomic and trophic groups (Table 18). For the numerator of the index, the percentage the trophic or taxonomic group contributes to the total of 21 species on Table 17 is used. An index value of 1.0 indicates that the taxonomic or trophic group was influenced by oil treatment on par with all species. An index of greater than 1.0 indicates that the group was more susceptible to the oil treatment than were all species. The appropriate percentages and ratios obtained are shown in Table 18. In terms of composition, polychaetes (index 1.41) and mollusks (index 1.09) were more severely influenced by the oil treatment than all species, and crustaceans (index 0.71) and "other" species (index 0.50) were less severely influenced by oiling. For the trophic groups, detritivores (index 1.45) and suspension feeders (index 1.54) and "other" species (index 1.25) were more severely influenced by the oil treatment, and carnivores (index 0.63) and herbivores (index 0.25) were less severely influenced by the oil treatment.

Petroleum Hydrocarbon Data

Time series data on total oil measured by IR, and initial and final concentrations of compound classes analyzed by capillary GC are shown in Figure 33. The compound class composition is identical to that previously described for the hard substrate experiments (Table 12).

Table 16. Hypothesis tests for density of primary species in commercial clam bed experiment at Discovery Bay during a 3-month period in 1980 (spring-summer, tide, oil).

	PROBABILITY FOR ERROR IN REJECTING THE HYPOTHESIS	
PRIMARY SPECIES	Tide Level[1]	Oil[2]
MOLLUSKS		
Mysella tumida	0.000*	0.000*
Protothaca staminea	0.054	0.014*
Lacuna sp.	0.002*	0.700
CRUSTACEANS		
Corophium ascherusicum	0.115	0.134
Photis brevipes	0.082	0.561
Leptochelia dubia	0.000*	0.000*
POLYCHAETES		
Armandia brevis	0.000*	0.000*
Exogone lourei	0.000*	0.018
Platynereis bicanaliculata	0.004*	0.540
Polydora socialis	0.003*	0.135

[1] The tide level hypothesis is: Density in trays at MLLW is equal to density in trays at +2' above MLLW.

[2] The oil hypothesis is: Density in trays receiving oil treatment is equal to density in trays not receiving oil treatment.

* We reject the indicated hypothesis with a maximum real probability for error of 10%.

Table 17. Species with trophic designation for which statistically significant (P = 0.05) oil treatment effects were computed.[1]

TAXONOMIC GROUP/SPECIES	TROPHIC GROUP[2]
POLYCHAETES	
Armandia brevis	detritivore
Axiothella rubrocincta	detritivore
Boccardia proboscidea	-
Exogone lourei	detritivore
Hemipodus borealis	carnivore
Notomastus (*Clistomastus*) *tenuis*	detritivore
Owenia fusiformis	detritivore
Protodorvillea gracilis	carnivore
Spio filicornis	detritivore
CRUSTACEANS	
Gnorimosphaeroma o. oregonensis	herbivore
Hemigrapsus nudus	carnivore
Leptochelia dubia	detritivore
Photis brevipes	suspension
Upogebia pugettensis	detritivore
MOLLUSKS	
Caecum occidentale	-
Macoma sp.	detritivore
Mysella tumida	suspension
Protothaca staminea	suspension
Transenella tantilla	suspension
OTHER SPECIES	
Leptosynapta clarki	-

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[1] Statistical significance computed from analysis of variance. The number of analyses precludes rigorous statistical evaluation. See methods and text for explanation. Experiment was at Discovery Bay during a 3-month spring-summer period, 1980.

[2] Trophic classification from Simenstad et al. (1979).

Table 18. Contribution to taxonomic and trophic groups overall and in terms of significant oil treatment effects.[1, 2]

TAXONOMIC/TROPHIC GROUP	PERCENT CONTRIBUTION Oil Treatment	 Overall	Ratio
TAXONOMIC			
Polychaetes	45	32	1.41
Crustaceans	25	35	0.71
Mollusks	25	23	1.09
Other Species	5	10	0.50
TROPHIC			
Detritivores	45	31	1.45
Carnivores	15	24	0.63
Suspension Feeders	20	13	1.54
Herbivores	5	20	0.25
Other Species	15	12	1.25

[1] The index of severity is computed as follows: Percentage contribution of taxon or trophic group to those species having "significant" oil treatment effects (Table 17) is divided by the percentage contribution of the taxon to numbers of individuals as a whole.

[2] Based on a 3-month experiment at Discovery Bay during a spring-summer period, 1980.

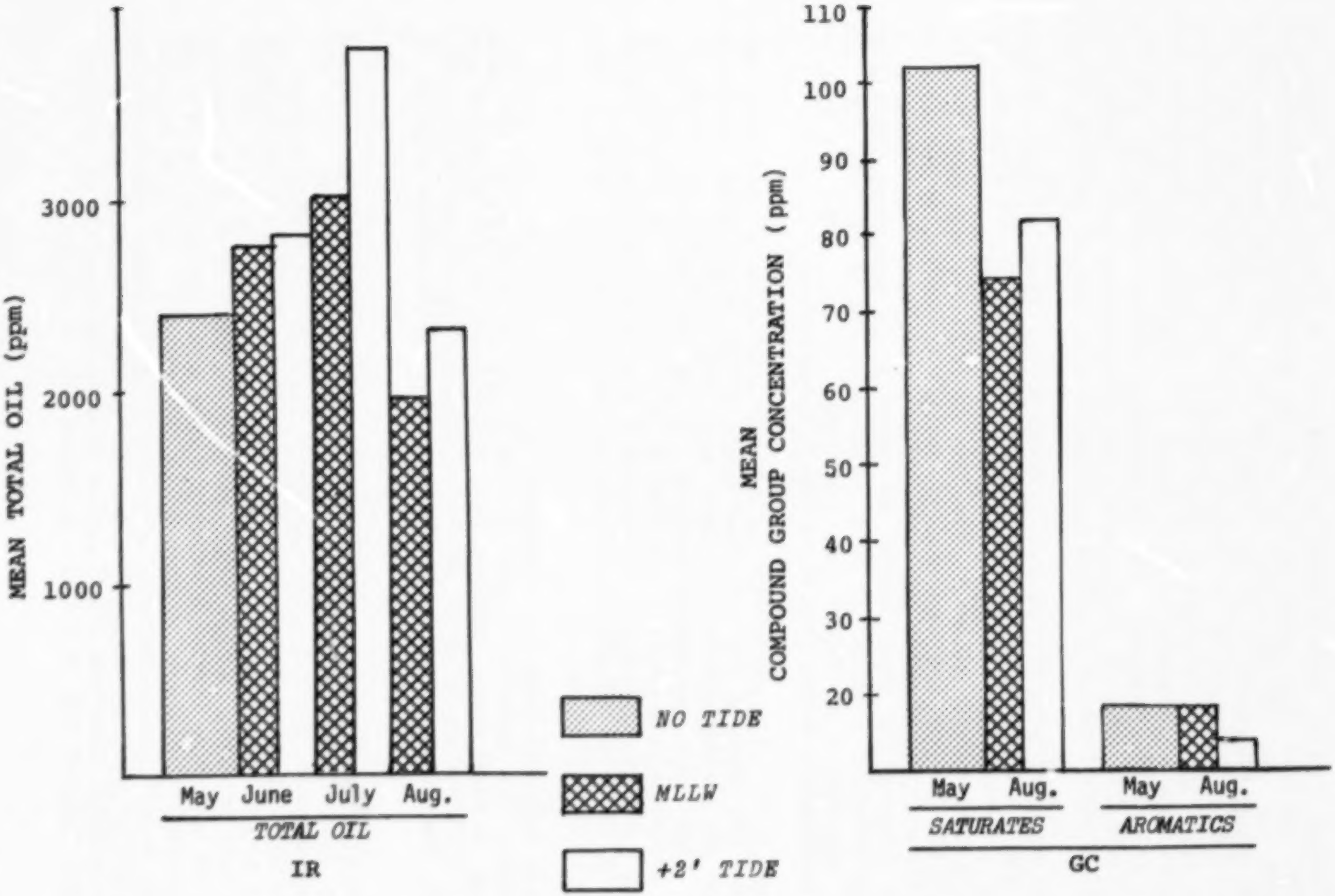

Figure 33. Time series of total oil and analyzed saturate and aromatic compounds in commercial clam bed recovery experiment at Discovery Bay, May through August, 1980 (IR = infrared spectrophotometry; GC = capillary chromatography).

The mean initial concentration of total oil in this experiment (nearly 2500 ppm) was more than twice as high as the equivalent summer experiment at Sequim Bay, and 400 ppm higher than the fall and long-term experiments in that group (Vanderhorst et al., 1980). Final concentrations in total oil were 80% at MLLW and 95% at +2' above MLLW of the initial concentrations (Figure 33). This is in sharp contrast to the findings for Sequim Bay and Protection Island sediments in which total oil concentrations were reduced by half in a similar period. The intermediate concentrations in June and July (Figure 33) indicate higher than initial concentrations. These concentrations were based on a single core analysis at each of the tide levels.

The analyzed saturate compound concentrations shown in Figure 33 were also higher initially than for previous experiments, and the loss was less over the three-month period (26% for MLLW, and 20% for +2'). In Sequim Bay and Protection Island sediments, the loss of saturates was about half in three months and was not dependent on initial concentration for rate of loss. The analyzed aromatic compounds had an initial mean concentration of 17 µg/g. During the three months, no reduction in mean concentration of analyzed aromatics occurred at MLLW. The reduction at +2' was 20%. In earlier experiments at Sequim Bay (Vanderhorst et al., 1980) the loss of analyzed aromatics ranged from 80 to 92% in three months.

Mean total oil concentrations at the conclusion of the clam bed experiment are given by the tide level and vertical core stratum, in Figure 34. For oil-treated sediments, higher mean concentrations are shown for the +2' tide level as compared to MLLW. Higher concentrations are shown for the bottom half of cores as compared to the top half. In contrast, the untreated sediments show higher background CCl_4 extractable organic concentrations at MLLW as compared to +2' and in the top half of cores as compared to the bottom half.

"Main effects" from oil treatment, tide level effect, and vertical core strata, are shown in Figure 35. Analyses of variance of the data resulting in these means indicate that the effects of oil treatment and vertical distribution in cores were statistically significant ($P = 0.05$), and that the effect of tide level was not a statistically significant effect.

The tide level and vertical core stratum distribution of analyzed saturate and aromatic compounds in cores is shown in Figure 36. For saturate compounds in oil-treated sediments, the tide level distribution is not consistent in the top and bottom half of cores. For the bottom half of cores, a higher concentration is shown at +2' as compared to MLLW, and for the top half of cores, a higher concentration is shown at MLLW as compared to +2'. At both tide levels, however, a higher mean concentration is shown in the bottom half of cores as compared to the top half. Analyzed aromatic compounds in oil-treated sediments also show slightly higher concentrations in the bottom half of cores as compared to top. The concentration of analyzed aromatic compounds are higher at MLLW in the top half of cores and about equal between tide levels in the bottom half of cores.

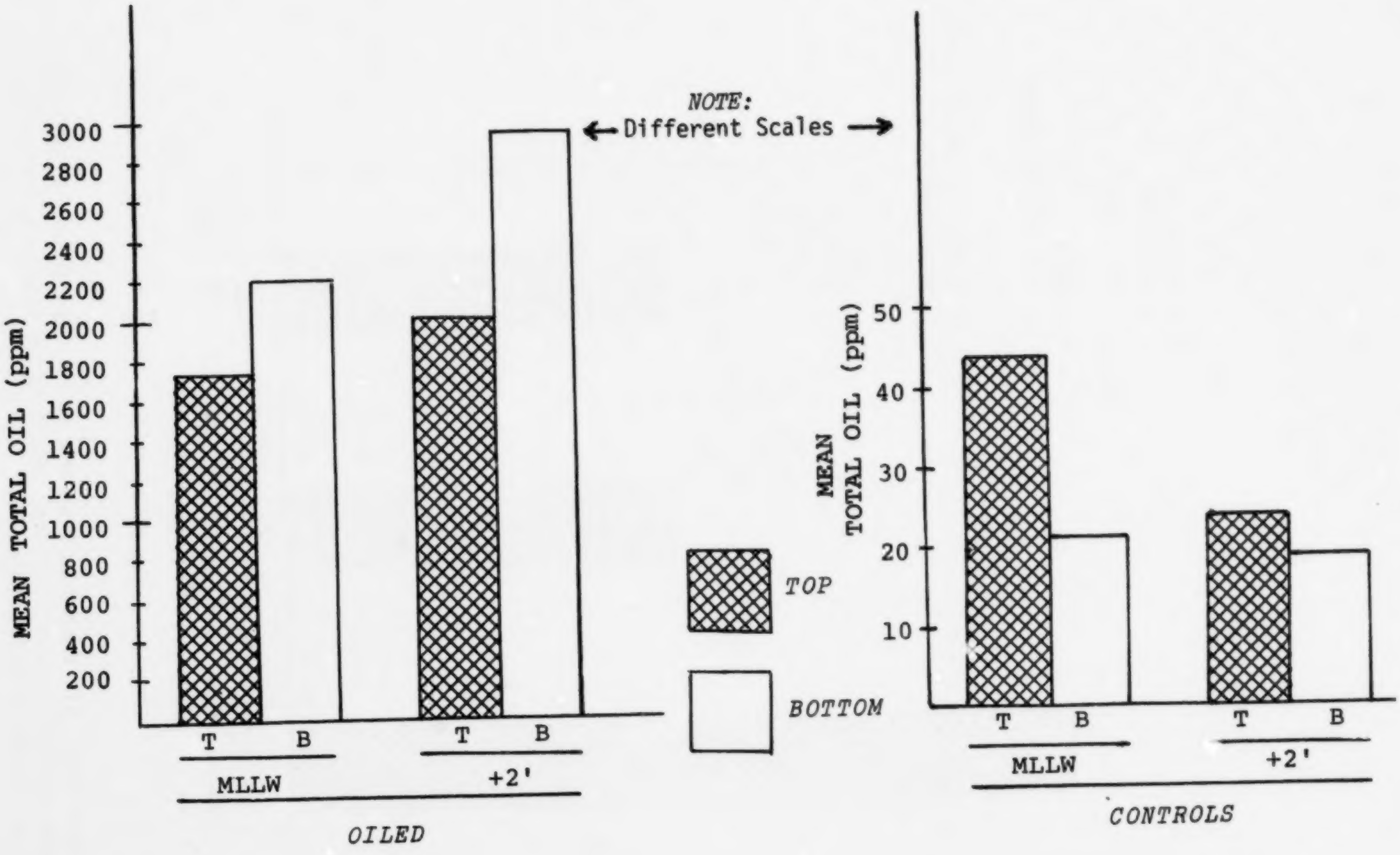

Figure 34. Vertical stratification of total oil concentration in cores for commercial clam bed recovery experiment at Discovery Bay, spring-summer season, 1980. Samples were taken in August 1980 at the conclusion of the experiment and measured using infrared spectrophotometry (Top = upper 5 cm of core; Bottom - remainder of core).

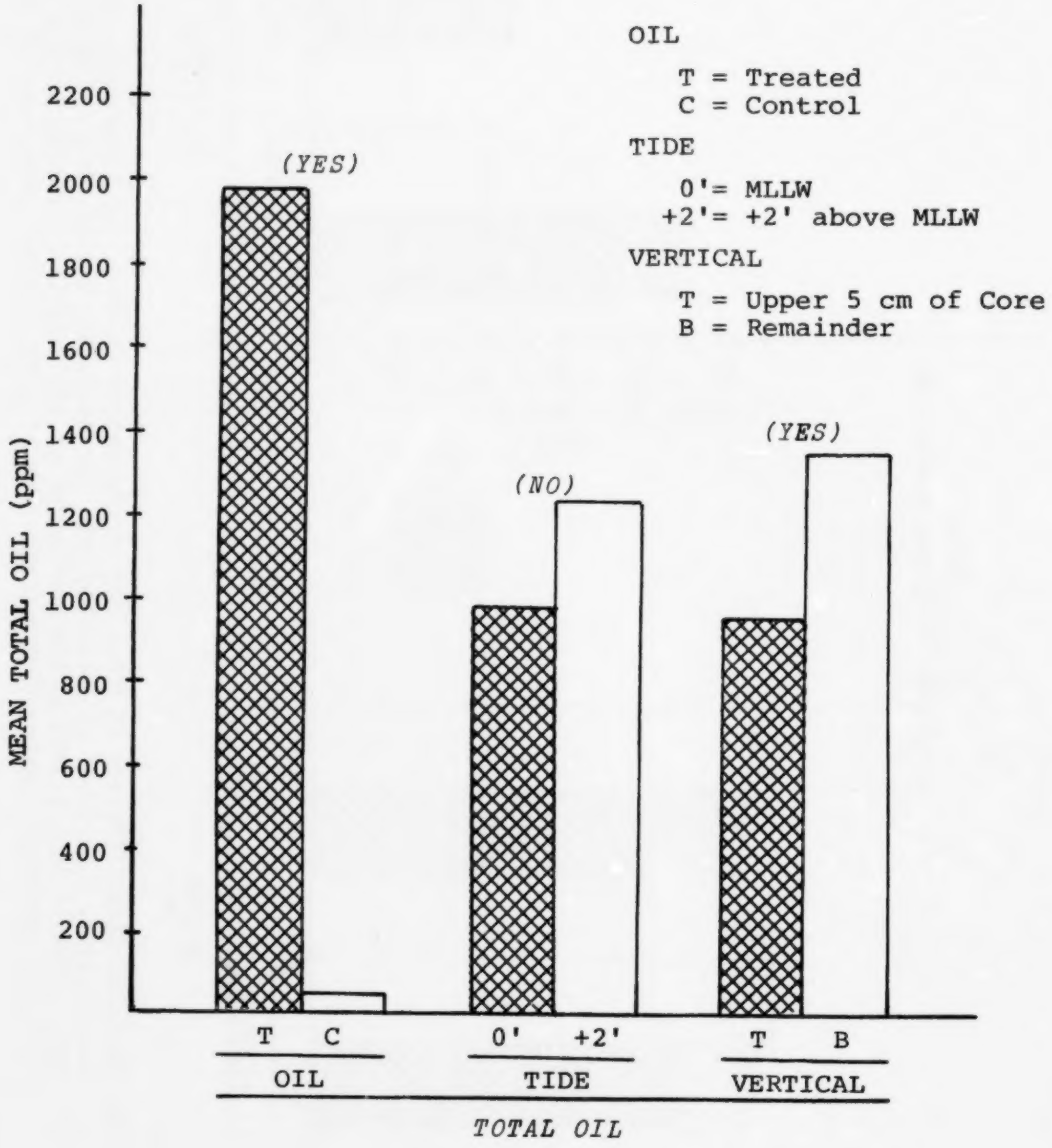

Figure 35. Mean total oil concentration due to oil treatment, tide level, and vertical stratification. Word in parentheses indicates statistical significance.

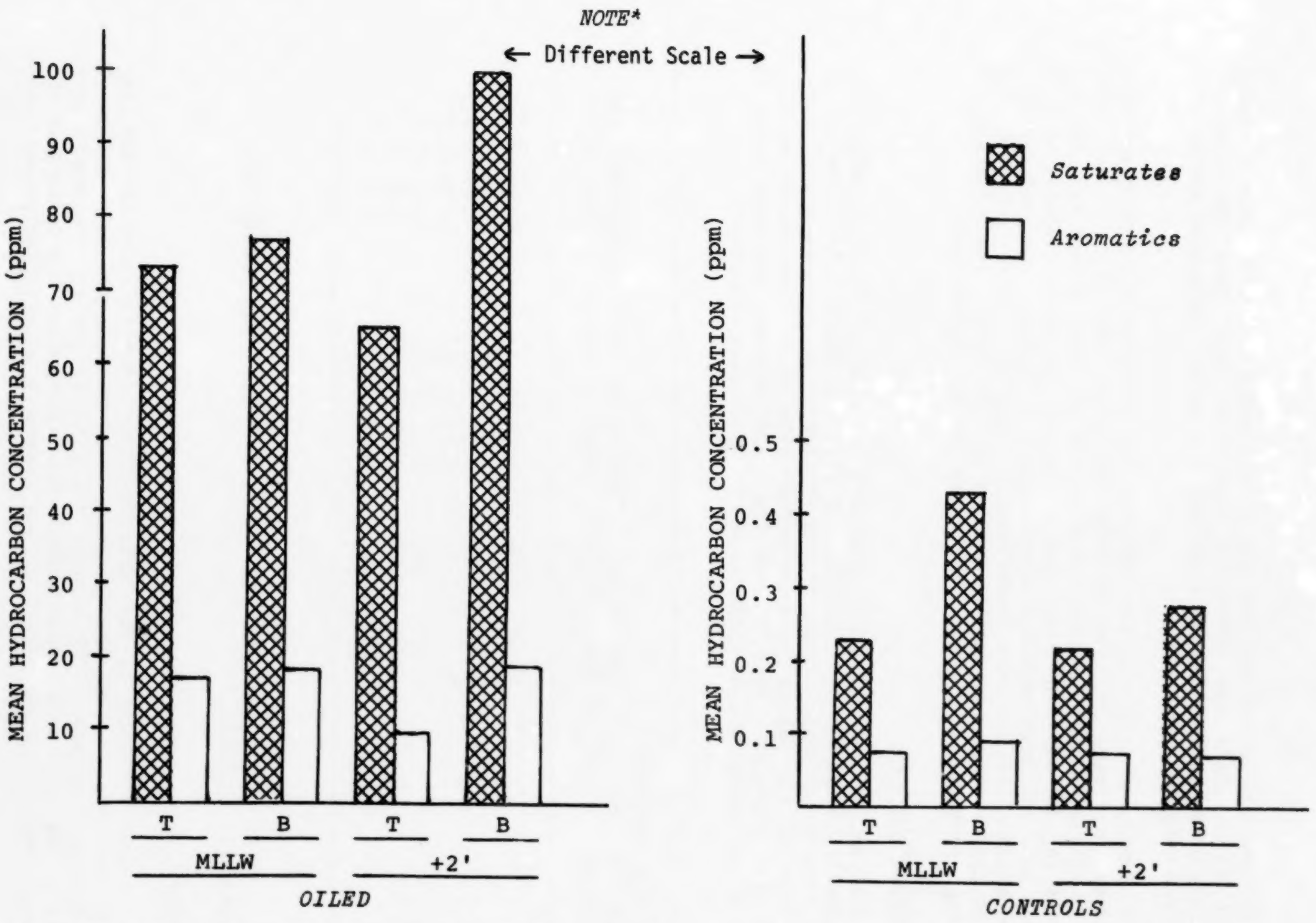

Figure 36. Vertical stratification of analyzed saturate and aromatic compound classes in relation to tide level and treatment from commercial clam bed experiment at Discovery Bay, spring-summer season, 1980. Saturate and aromatic hydrocarbons measured by capillary gas chromatography.

It should be noted that the data on untreated controls shown in Figure 36 are on a scale two orders of magnitude lower than the data for oil-treated sediments. The data reflect the normal background variability for these compound classes.

A summary of analysis for saturate and aromatic compound class data is shown in Figure 37. For saturate compounds, the effect of treatment was, of course, significant (P = 0.05). The slightly higher mean concentrations for the +2' tide level and bottom half of cores compared to MLLW and top half of cores were not significantly different. Analyzed aromatic compound concentrations were significantly higher in oil-treated and bottom half of cores compared to unoiled and top half of cores. The difference in mean concentrations at the two tide levels was not significant.

Sediment Grain Size

Analyses of sediment grain size were performed on 11 cores taken from experimental trays at the Discovery Bay Clam Bed (MLLW). Additionally, 10 cores from experimental trays at the Protection Island site and 12 cores from trays at the Sequim Bay experimental site (MLLW, Vanderhorst et al., 1980) were analyzed. Results from these analyses are tabulated below in terms of percentage weight contribution by grain size fractions:

GRAIN SIZE FRACTION SCREEN SIZE (mm) GREATER THAN	SEQUIM BAY Mean (S.D.)	PROTECTION ISLAND Mean (S.D.)	DISCOVERY BAY Mean (S.D.)
5.66	11.18 (3.49)	0.87 (0.53)	52.53 (3.33)
2.00	19.54 (3.03)	3.59 (0.83)	15.08 (1.43)
1.00	17.35 (3.96)	3.27 (1.00)	7.99 (0.73)
0.50	25.38 (2.72)	6.53 (1.58)	10.30 (0.71)
0.125	24.09 (5.10)	83.71 (2.99)	12.30 (0.89)
0.063	2.05 (0.64)	1.83 (0.27)	1.32 (0.17)
PAN	0.40 (0.26)	0.21 (0.05)	0.47 (0.07)

EFFECTS OF OIL AND KEY SPECIES REMOVAL ON HARD SUBSTRATE COMMUNITIES AND COMMUNITY RECOVERY

A Perspective

The data presented in this section differ from those in the two preceding sections in that bricks were allowed to colonize for a period of nine months (September 1979 through May 1980) before any treatments were applied. The three independent experiments reported had the primary objectives of evaluating the effect of oil treatment on existing associations and the recovery of those associations, and the effect of a differential in grazing pressure on the associations. For experiments in the preceding sections, the nature of the effects due oil treatment was clear, i.e., the effect of the oil treatment, if any, was to alter the suitability of the substrate itself. This was measured by comparing treated and untreated substrates given equal field exposure conditions (through randomization of multiple units, Figure 37). In an attempt to distinguish between effects on the associations from the treatments, and effects related to a differential in recovery between treated and untreated bricks, the three experiments reported in this section were treated in analyses of variance. Using duration of field exposure after oil or grazer treatment as a "treatment" in the experimental design model:

$$Y_{ijkl} = D_i + O_j + G_k + T_l + E$$

where Y = the response variable magnitude;

D = the main effect due to duration of field exposure (i = 0, 5, or 30 days);

O = the main effect due to oil treatment (j = oil-treated or untreated);

G = the main effect due to grazers (k = limpets stocked or removed from associations);

T = the main effect due to field exposure tide level (l = MLLW or +2' above MLLW); and

E = random error.

The response variables evaluated in analyses of variance were of the same types as those in the other hard substrate experiments, and, in addition, included dry weight biomass of algae. This latter response variable was of particular interest because of an expected relationship between algae and grazers.

The species composition for these experiments overall is shown in Table 19. All experiments were conducted at Sequim Bay. For the present experiments, a total of 95 species are shown in Table 19. For the monthly experiments overall, including the site experiments, a total of 79 species were shown. Thus, the number of species for these precolonization experiments is higher than the accumulated total for the 12 other experiments. In fact, this is a very conservative estimate of the true difference in structure because the aggregate sample size contributing to the 95 species in the present experiments was a total of 120 bricks giving an average of 0.8 species per brick, and the aggregate sample size in the 12 monthly experiments was 840 bricks for an average of 0.09 species per brick. This approximate order of magnitude difference in numbers of species per brick reflects the importance of community structure in the present experiments since the total available colonization time (10 months) and the specific 10-month period were identical for each of the two cases.

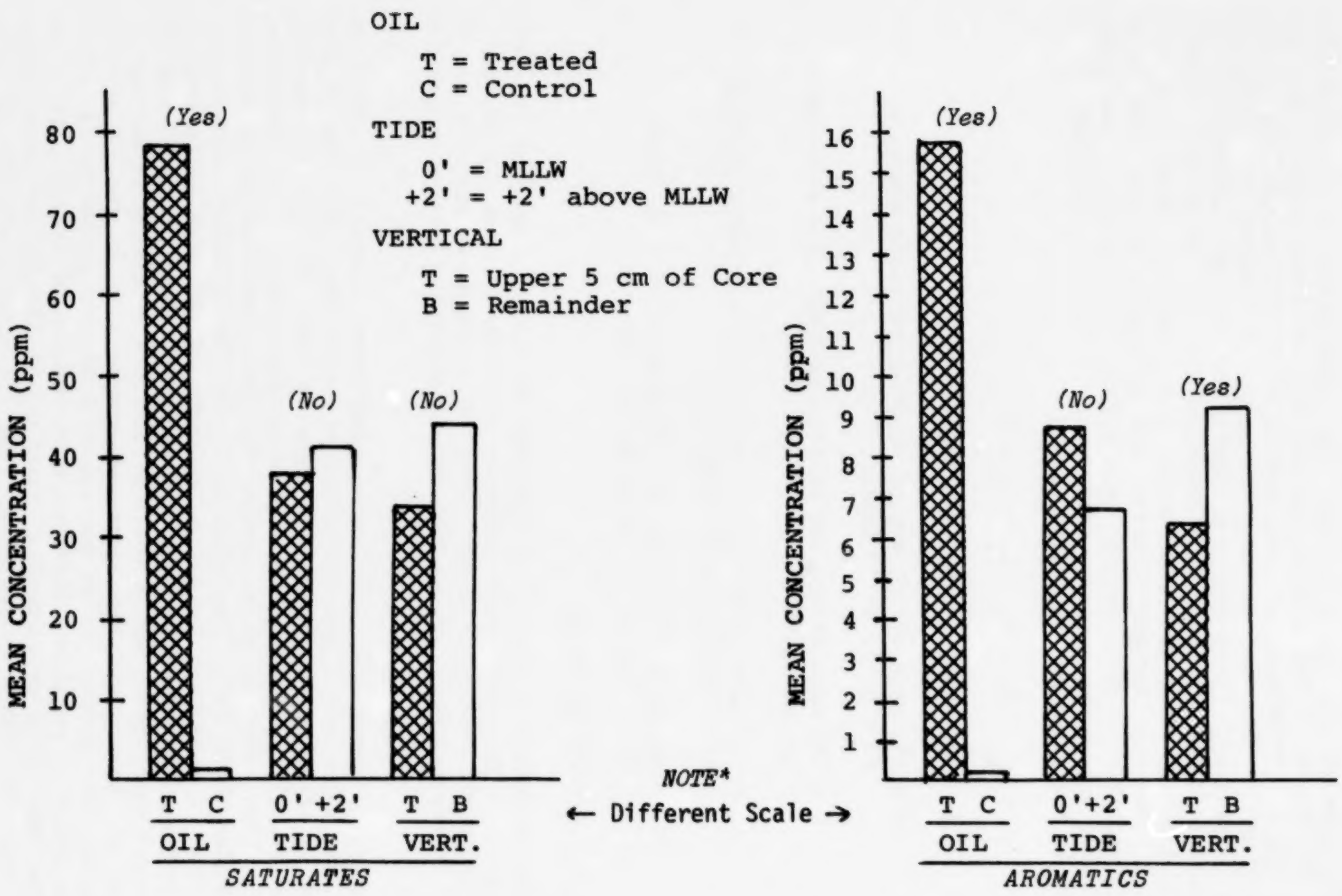

Figure 37. Saturated and aromatic compounds from commercial clam bed experiment at Discovery Bay, spring-summer season, 1980. Samples were taken in August 1980 and measured using capillary gas chromatography. Word in parentheses indicates statistical significance.

Table 19. Taxonomic and trophic composition in grazer experiments.[1]

SPECIES/TAXONOMIC GROUP	TROPHIC GROUP[2]
POLYCHAETES	
Anaitides groenlandica	carnivore
Anaitides williamsi	carnivore
Anaitides sp.	carnivore
Armandia brevis	detritivore
Axiothella rubrocincta	detritivore
Boccardia proboscidea	suspension
Capitella capitata	detritivore
Cirratulus cirratus	detritivore
Dorvillea sp.	carnivore
Eulalia nigrimaculata	carnivore
Eulalia sp.	carnivore
Exogone lourei	detritivore
Halosynda brevisetosa	carnivore
Harmothoe imbricata	carnivore
Lumbrinereis sp.	herbivore
Maldanidae undet.	detritivore
Nephthys caecoides	carnivore
Nereis vexillosa	herbivore
Nothria elegans	herbivore
Opheliidae undet.	detritivore
Ophiodromus pugettensis	carnivore
Phyllodocidae undet.	carnivore
Platynereis bicanaliculata	herbivore
Polychaeta undet.	varied
Polydora socialis	detritivore
Polydora sp.	detritivore
Protodorvillea gracilis	carnivore
Spio filicornis	detritivore
Spionidae undet.	detritivore
Thelepus crispus	detritivore
Thelepus sp.	detritivore
CRUSTACEANS	
Amphipoda undet.	varied
Ampithoe simulans	herbivore
Ampithoe sp.	herbivore
Anonyx sp.	detritivore
Aoroides columbiae	detritivore
Balanus cariosus	suspension
Balanus glandula	suspension
Balanus sp.	suspension

Table 19. (Continued)

SPECIES/TAXONOMIC GROUP	TROPHIC GROUP[2]
CRUSTACEANS (Continued)	
Caprella laeviuscula	herbivore
Caprella sp.	herbivore
Ceradocus spinicaudus	detritivore
Corophium sp.	detritivore
Exosphaeroma sp.	herbivore
Fabia subquadrata	-
Hippolyte sp.	carnivore
Idothea wosenesenskii	herbivore
Ischyrocerus sp.	suspension
Jassa falcata	detritivore
Leptochelia dubia	detritivore
Leptochelia sp.	detritivore
Maera sp.	detritivore
Melita californica	detritivore
Nebalia pugettensis	suspension
Pagurus hirsutiusculus	detritivore
Pagurus sp.	detritivore
Parallorchestes ochotensis	detritivore
Paraphoxus sp.	detritivore
Photis sp.	suspension
Phoxocephalidae undet.	detritivore
Pinnixia *faba*	other
Pinnixia tubicola	other
Pinnixia schmitti	other
Pinnixia sp.	other
Pontogeneia inermis	detritivore
Pugettia gracilis	herbivore
MOLLUSKS	
Acmaea digitalis	herbivore
Acmaea persona	herbivore
Acmaea sp.	herbivore
Alvania sp.	herbivore
Bittium sp.	carnivore
Caecum occidentale	-
Cyanoplax hartwegii	herbivore
Lacuna sp.	herbivore
Littorina sp.	herbivore
Margarites pupillus	herbivore
Margarites sp.	herbivore
Mitrella sp.	-
Mopalia muscosa	carnivore
Mysella tumida	suspension
Mytilus edulis	suspension

Table 19. (Continued)

SPECIES/TAXONOMIC GROUP	TROPHIC GROUP[2]
MOLLUSKS (Continued)	
Odostomia sp.	-
Protothaca staminea	suspension
Searlesia dira	carnivore
Searlesia sp.	carnivore
Transennella tantilla	suspension
OTHER SPECIES	
Amphipholis sp.	detritivore
Emplectonema gracile	carnivore
Evasterias troschelli	carnivore
Leptasterias hexactis	carnivore
Oligocottus sp.	carnivore
Paranemertes peregrina	carnivore
Paranemertes sp.	carnivore
Pholis laeta	-
Pholis sp.	-

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[1] This composition results from analysis of all bricks used in the grazer experiments (N = 12). Bricks had been field exposed at Sequim Bay from September 1979 through June 1980. Both MLLW and +2 MLLW tide levels are represented.

[2] Trophic categories from Simenstad et al. (1980); (-) indicates no data.

Thus, although the compositional list (Table 19) is simple in terms of other sampled rocky intertidal communities (Nyblade, 1979) reported in excess of 900 species for a combination of several Strait of Juan de Fuca stations), effects from treatments resulting in a reduction of numbers of species or individuals in the present experiments will reflect a breakdown in community complexity, a stated task objective.

Taxonomic and Trophic Composition

A breakdown of the data on Table 19 indicates the 95 species were: 31 polychaetes (32%); 35 crustaceans (38%); 20 mollusks (21%); and nine species not belonging to the major taxonomic groups (9%). For trophic categories overall (using Simenstad et al. (1979) for classification), the 95 species were: 29 detritivores (31%); 23 carnivores (24%); 11 suspension-feeders (11%); 20 herbivores (21%); and 12 species not fitting any of these categories (13%).

The taxonomic groups differed in trophic composition: among the polychaetes, 13 species (42%) were detritivores; 12 species (39%) were carnivores; four species (13%) were herbivores; and one species each (3% each) were suspension-feeders or species not fitting the trophic categories. For crustaceans, 16 species (44%) were detritivores; seven species (19%) were herbivores; six species each (17% each) were suspension-feeders or did not fit the trophic classification; and only one species (3%) was a carnivore. Mollusks had no detritivores; nine herbivores (45%); four each of carnivores and suspension-feeders (20% each); and three species not fitting the trophic categories (15%). The species outside the major taxonomic categories included six carnivores (67%); two species outside the trophic classification (22%); one detritivore (11%); and no suspension-feeders or herbivores.

Treatment Effects - Field Exposure Time, Oil and Grazers

The main effects means for numbers of species in taxonomic groups related to duration of post-treatment period are shown in Figure 38. A statement of statistical significance (P = 0.05) of differences between these means is indicated in parentheses over each comparison. Total number of species and numbers of species in each of the taxonomic groups except crustaceans were less in the 0-day field exposure experiment than in the 30-day field exposure. For polychaetes and total species the differences between field exposure periods were significant and indicate colonization during the 30-day post-treatment period. The intermediate 5-day field exposure experiment showed fewer mean number of species than 0-day field exposure for total species, crustaceans, and mollusks. Although the statistical analyses used do not permit assignment of probability for significance of these differences, they do not appear to be of a magnitude which would be deemed significant if treated independently.

Mean number of species per brick for oil and grazer main effects are shown in Figure 39. Statistical significance of differences between group means (P = 0.05) is shown in parentheses above the means compared.

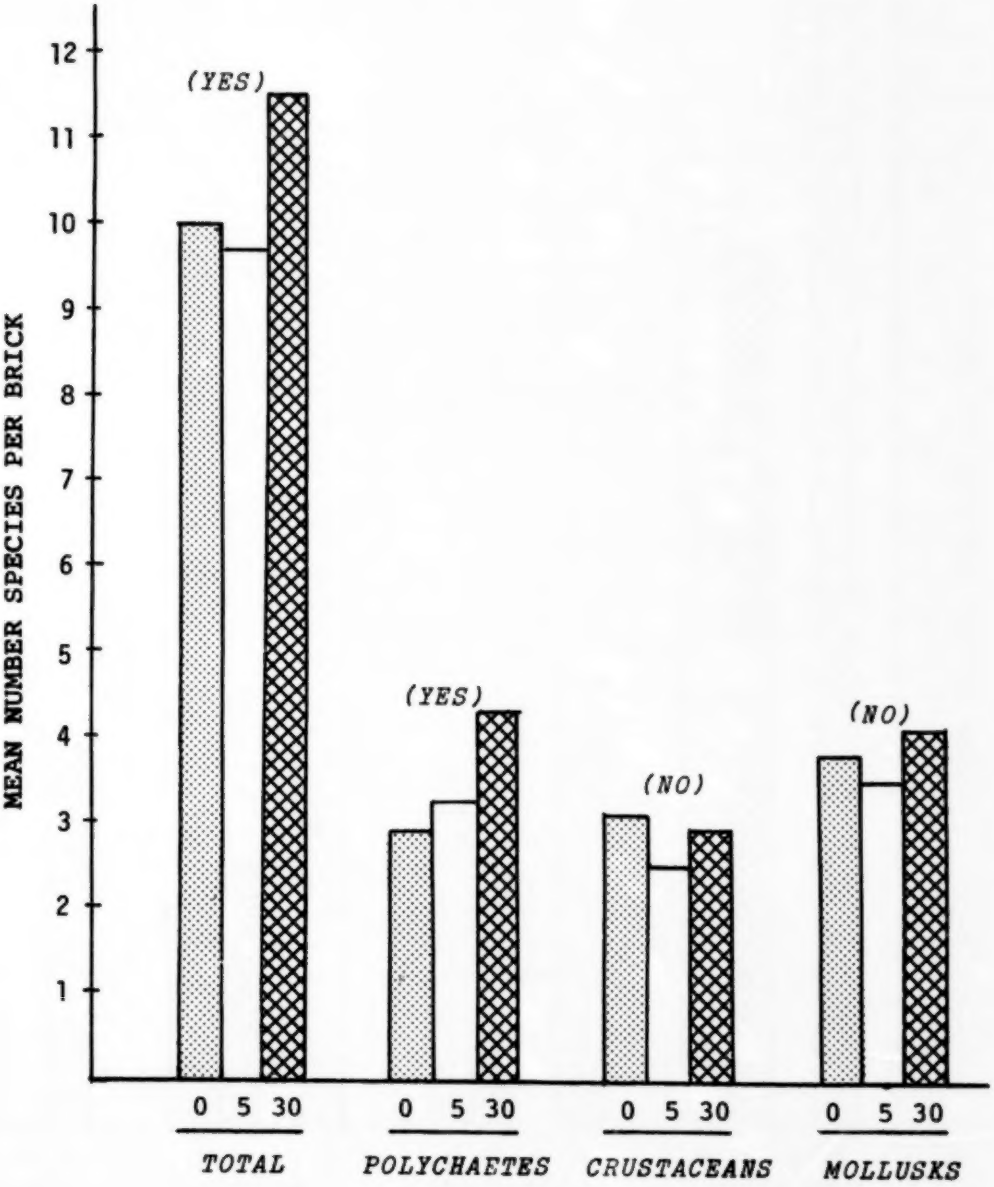


Figure 38. Mean number of species per brick related to duration of experiment. Data summarized over two tide levels (MLLW and +2' above MLLW); two oil treatments (oiled and unoiled); and two grazer treatments (limpets stocked and limpets removed). (N = 120 bricks.)

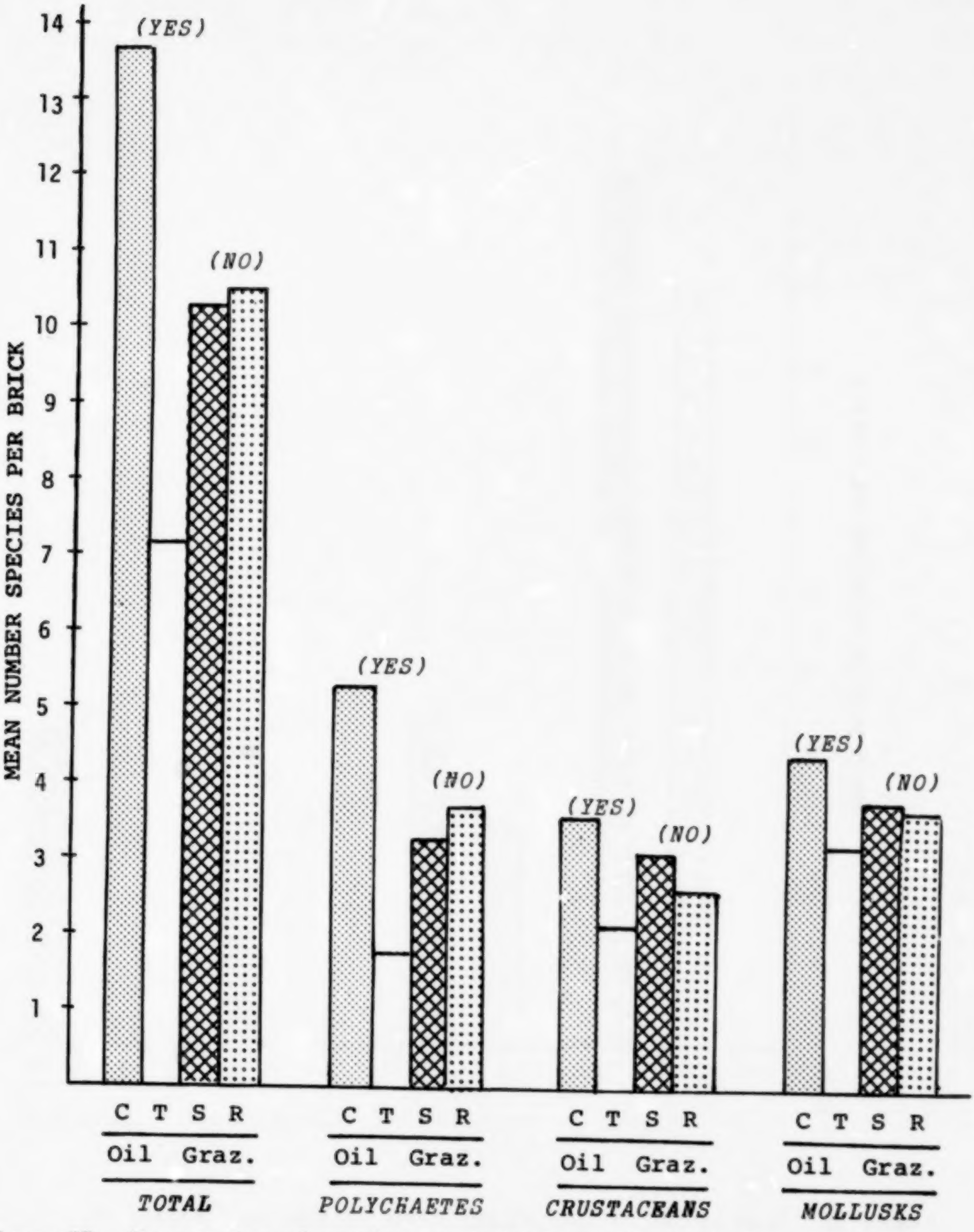


Figure 39. Mean number of species per brick summarized by main effects of oil treatment and grazer treatment of experiments at Sequim Bay, May and June, 1980 (C = control; T = treated; S = limpets stocked; R = limpets removed). (N = 120 bricks.) Word in parentheses indicates statistical significance.

Significant effects on numbers of species due to oil treatment (C = control; T = oil-treated, Figure 39) for each of the taxonomic groups and total number of species were demonstrated. In no case was a statistically significant effect demonstrated for the grazer treatment (S = limpets stocked; R = limpets removed).

The mean number of species for tide level main effects are shown in Figure 40. There is a slightly higher mean number of species per brick in all categories at the MLLW tide level. In no case was the difference in number of species per brick due to tide level deemed significant.

A comparison of mean numbers of individuals per brick within taxonomic and trophic categories related to duration of field exposure is shown in Figure 41. A statement of statistical significance ($P = 0.05$) is shown in parentheses above each comparison of means. There was a higher mean density at 30 days post-treatment compared to immediately post-treatment for each of the taxonomic and trophic groups. Differences between mean densities due to duration of period post-treatment were demonstrated to be statistically significant for crustaceans, mollusks, herbivores, and suspension-feeders. It is of interest that the largest mean difference in group density (detritivores, about 40 individuals per brick difference) was not significant. This is either due to high error variance or some other factor in the model contributing to large differences in the number of detritivores. The intermediate (5-day post-treatment) mean numbers of individuals per brick were higher than immediate post-treatment for crustaceans, mollusks, herbivores, and detritivores. Although the significance of the differences in these means is not tested in the model, it appears that the magnitude of difference in mean number of mollusks and herbivores would be significant if independently tested.

The mean densities for main effects due to tide level are shown in Figure 42. Statistically significant tide level effects were demonstrated for polychaetes, crustaceans, and mollusks (P, C, M, over taxonomic categories, Figure 42); and suspension-feeders and herbivores (S and H over trophic categories, Figure 42). Significantly higher densities at MLLW as compared to +2' above MLLW are shown for crustaceans, mollusks, and herbivores. Significantly higher densities at the +2' above MLLW tide level are shown for polychaetes and suspension-feeders. Tide level effects for carnivores and detritivores were not deemed significant. It is of interest that the mean difference for density of detritivores between tide levels is practically nil, and, thus, does not provide for the high nonsignificant mean differences related to duration seen earlier (Figure 41).

Main effects mean numbers of individuals per brick due to grazer stocking and removal are shown in Figure 43. Significant effects were demonstrated for differences in density of polychaetes, mollusks, and herbivores. The manipulated species (*Acmaea* spp.) belong to mollusk and herbivore groups. Thus, the significant difference indicating higher mean density on grazer-stocked versus grazer-removed (S versus R, Figure 43), are in part a reflection that the manipulation itself was detected by the sampling approach. In the case of polychaetes, a significantly higher

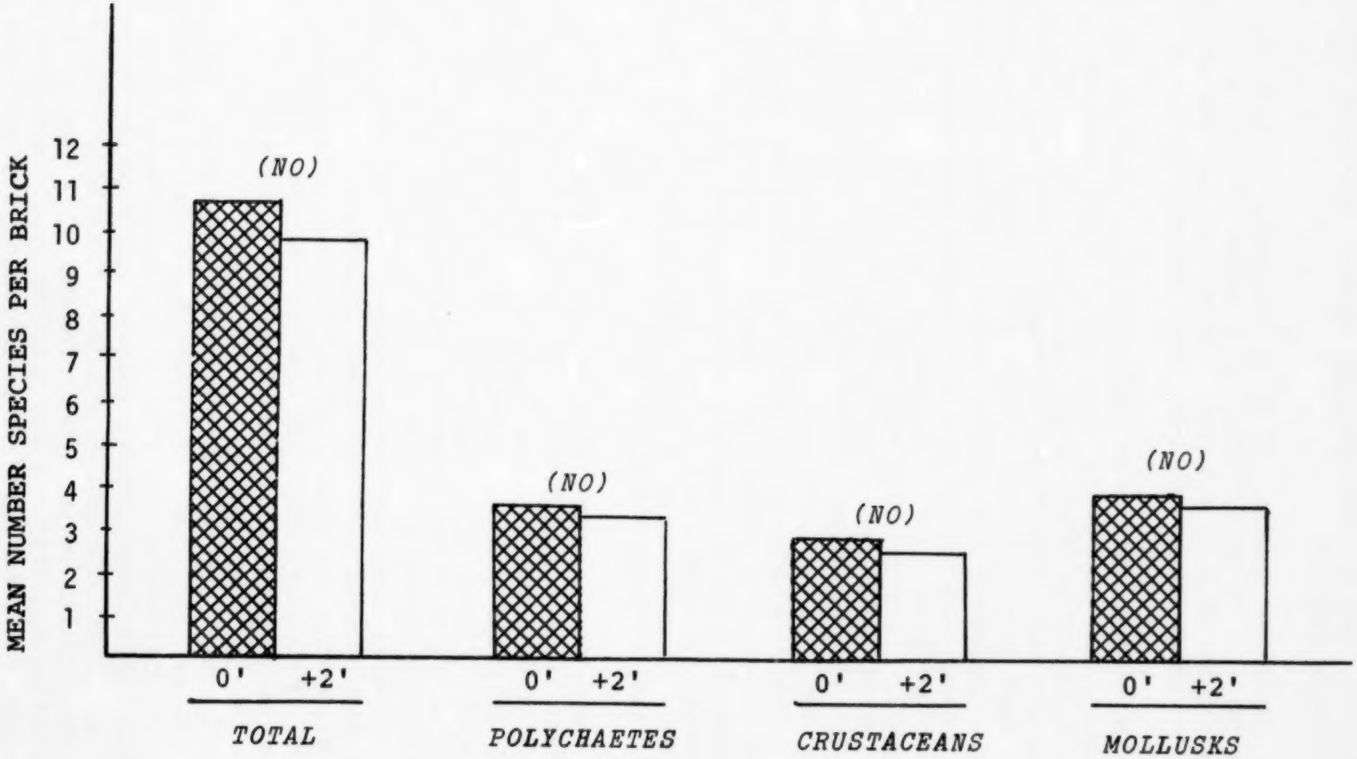


Figure 40. Mean number of species per brick of experiments conducted at Sequim Bay, May and June, 1980, related to tide level. Data summarized over two oil treatments (oiled and unoiled); two grazer treatments (limpets stocked and limpets removed); and 0-, 5-, and 30-day field exposure bricks (0' = MLLW; +2' = 2' above MLLW). (N = 120 bricks.) Word in parentheses indicates statistical significance.

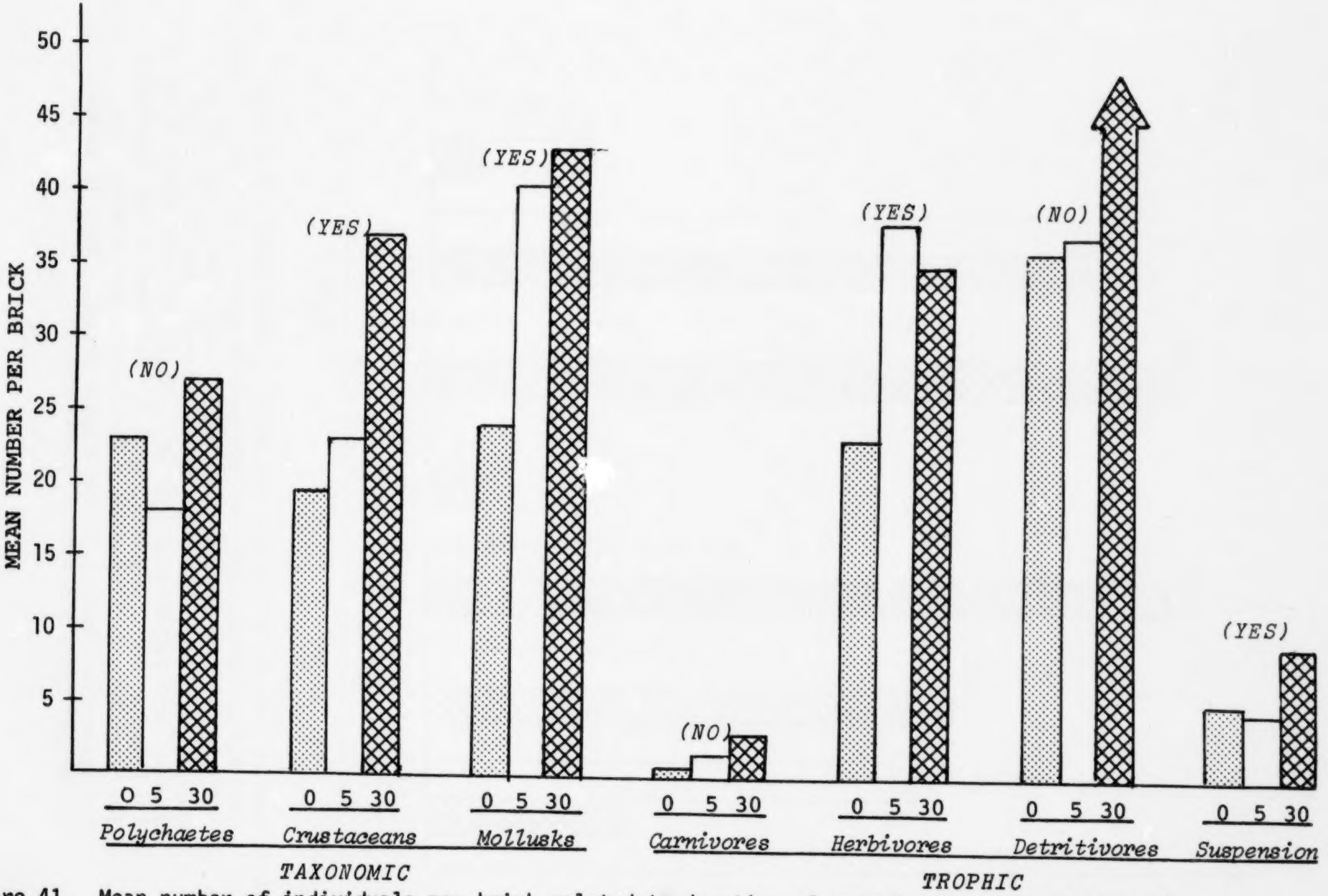


Figure 41. Mean number of individuals per brick related to duration of experiments at Sequim Bay, May and June, 1980. Field exposure main effects summarized over two treatments (oiled and unoiled); two tide levels (MLLW and +2' above MLLW); and two grazer treatments (limpets stocked and limpets removed). N = 120 bricks.) 0 = immediately post treatment; 5 = 5 days post-treatment; 30 = 30 days post-treatment.) Word in parentheses indicates statistical significance.

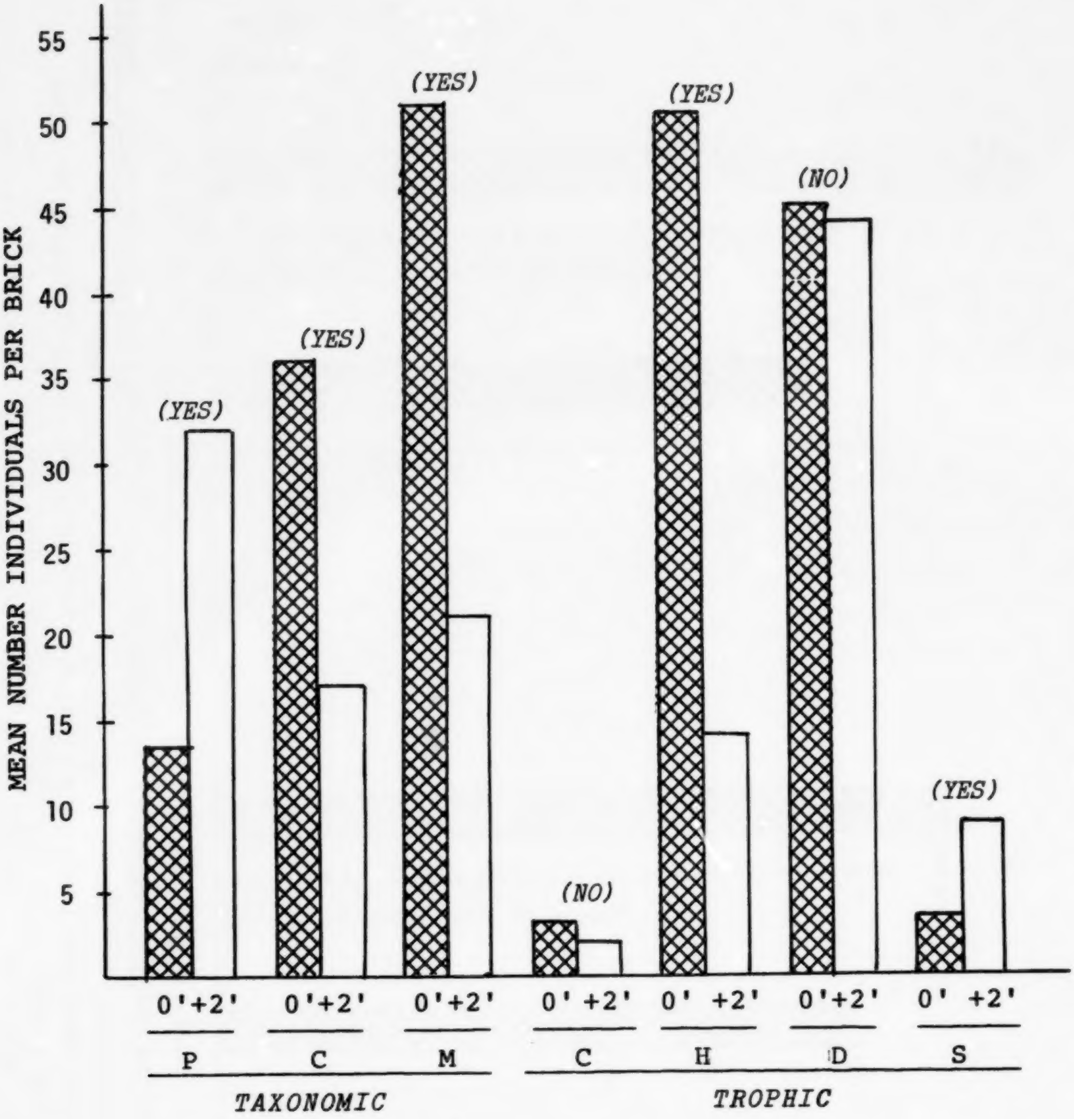


Figure 42. Mean number of individuals per brick of experiments at Sequim Bay, May and June, 1980, related to tide level. Data summarized over two oil treatments (oiled and unoiled); two grazer treatments (limpets stocked and limpets removed); and 0-, 5-, and 30-day field exposure periods (0' = MLLW; +2' = 2' above MLLW; P = Polychaetes; C - Crustaceans; M = Mollusks in the Taxonomic Groups and C = Carnivores; H = Herbivores; D = Detritivores; S = Suspension Feeders in the Trophic Group). Word in parentheses indicates statistical significance.

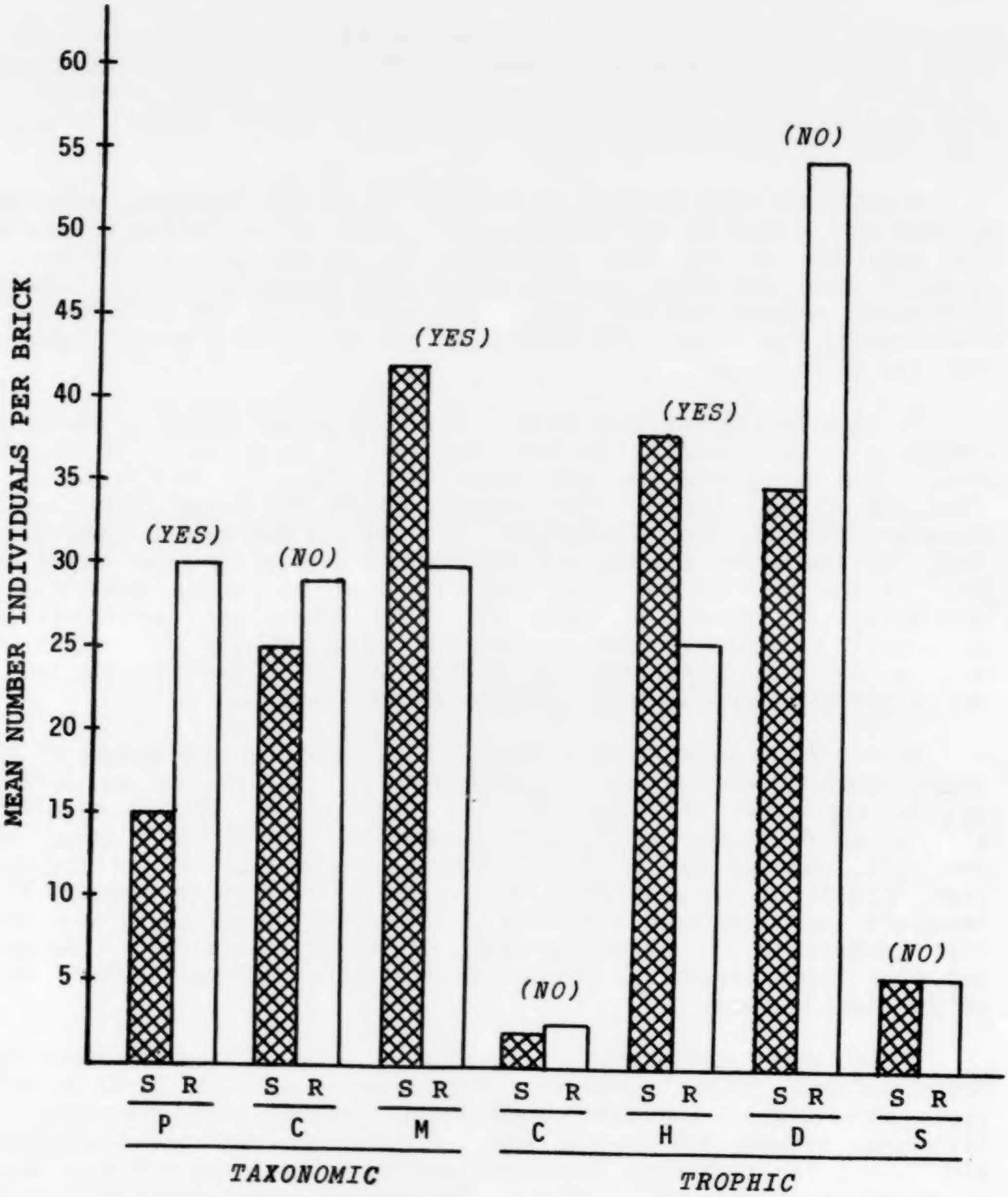


Figure 43. Mean number of individuals per brick of experiments at Sequim Bay, May and June, 1980, related to grazer treatment. Data summarized over two oil treatments (oiled and unoiled); two tide levels (MLLW and +2' above MLLW); and 0-, 5-, and 30-day field exposure periods (S = limpets stocked; R = limpets removed). (P = Polychaetes; C = Crustaceans; M = Mollusks in the Taxonomic Group and C = Carnivores; H = Herbivores; D = Detritivores; S = Suspension Feeders in the Trophic Group.) Word in parentheses indicates statistical significance.

density is shown for grazer-removed bricks (R, Figure 43). This may reflect competitive interaction between polychaetes and the limpets; however, the methods used do not allow such discrimination. Higher mean densities (not statistically significant differences) on grazer removal bricks are also shown for detritivores and crustaceans.

Significant main effects on density due to oil treatment were demonstrated within each of the taxonomic and trophic groups tested (Figure 44). The magnitude of the mean difference in density for detritivores far exceeded that for other trophic groups and taxonomic groups. The mean difference between control and oil-treated bricks for polychaetes and crustaceans, the major contributors to the detritivore group (Table 19), was also quite large.

To examine the possible effects from the grazer stocking and removal treatment in more detail, the herbivore species were subdivided into those which feed on microalgae and those which feed on macroalgae as per Simenstad et al. (1979). The composition of the algae itself was not measured; however, the sea lettuce, Ulva sp., a macroalgal type, clearly dominated the plant biomass and appeared to completely cover every brick used in the experiments. The composition of macroalgae and microalgae herbivores is shown in Table 20. The microalgae herbivores were principally mollusks and two crustaceans. They included the species which were manipulated, Acmaea spp., as well as several herbivorous snails. The macroalgae herbivores were polychaetes and crustaceans.

Main effects means from analysis of variance for dry weight of total algal biomass, microalgae herbivore density, and macroalgae herbivore density are shown in Figure 45. There were statistically significant effects on mean dry weight total algae due to duration (day) post-treatment, and due to the oil treatment. There was a higher dry weight algal biomass in the experiment 30 days post-treatment as compared to the immediate post-treatment experiment. There was a decreased dry weight algal biomass in oil-treated bricks as compared to controls. The grazer and tide level treatments did not result in significant effects on dry weight algal biomass.

There were statistically significant effects from duration (day) post-treatment, grazer stock-removal treatment, and tide level on microalgae herbivores. The tide level effect appears most important. The difference between oil-treated and control bricks was not statistically significant for microalgae herbivore density. There was a higher density of microalgae herbivores in the 30-day post-treatment experiment as compared to the immediate post-treatment experiment. There was a higher mean density of microalgae herbivores on bricks receiving a stock of limpets as compared to those where limpets were removed. There was a higher density of microalgae herbivores at MLLW as compared to +2' above MLLW.

There were significant differences in density for macroalgae herbivores due to day post-treatment, and due to the effect of oil treatment. For this group there was a lower density 30 days post-treatment as compared

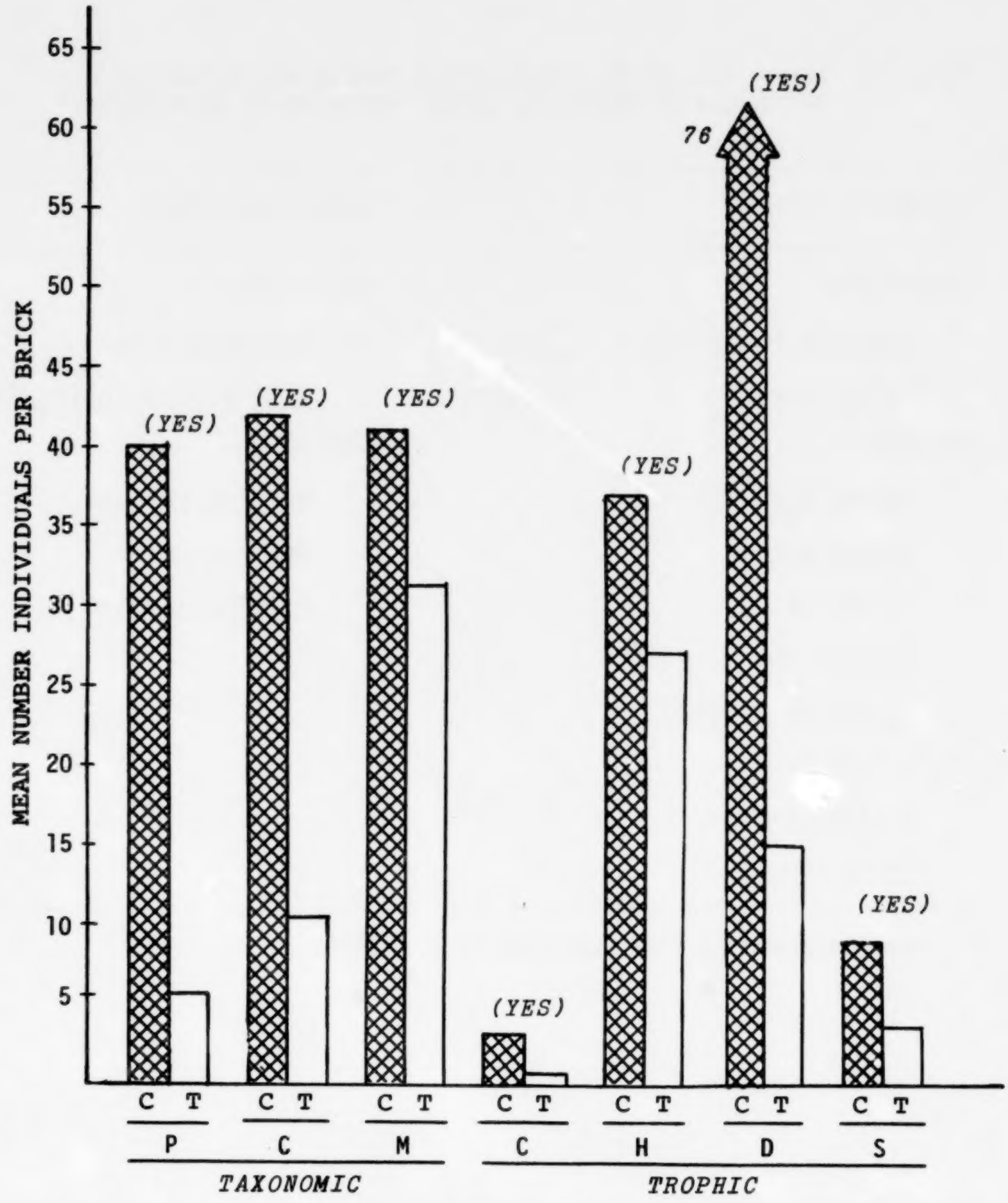


Figure 44. Mean number of individuals per brick of experiments at Sequim Bay, May and June, 1980, related to oil treatment. Data summarized over two tide levels (MLLW and +2' above MLLW); two grazer treatments (limpets stocked and limpets removed); and 0-, 5-, and 30-day field exposure periods (C = control; T = treated). P = Polychaetes; C = Crustaceans; M = Mollusks in the Taxonomic Groups and C = Carnivores; H = Herbivores; D = Detritivores; S = Suspension Feeders in the Trophic Group). Word in parentheses indicates statistical significance.

Table 20. Composition of herbivores which feed on microalgae and macroalgae in Sequim Bay grazer manipulation experiments.[1]

MICROALGAE FEEDERS	MACROALGAE FEEDERS
CRUSTACEANS	POLYCHAETES
Caprella laeviuscula	*Nereis vexillosa*
Caprella sp.	*Platynereis bicanaliculata*
MOLLUSKS	CRUSTACEANS
Acmaea digitalis	*Ampithoe simulans*
Acmaea persona	*Ampithoe* sp.
Acmaea sp.	*Pugettia gracilis*
Alvania sp.	
Cyanoplax hartwegii	
Lacuna sp.	
Littorina sp.	
Margarities sp.	

[1] Trophic designation from Simenstad et al. (1979).

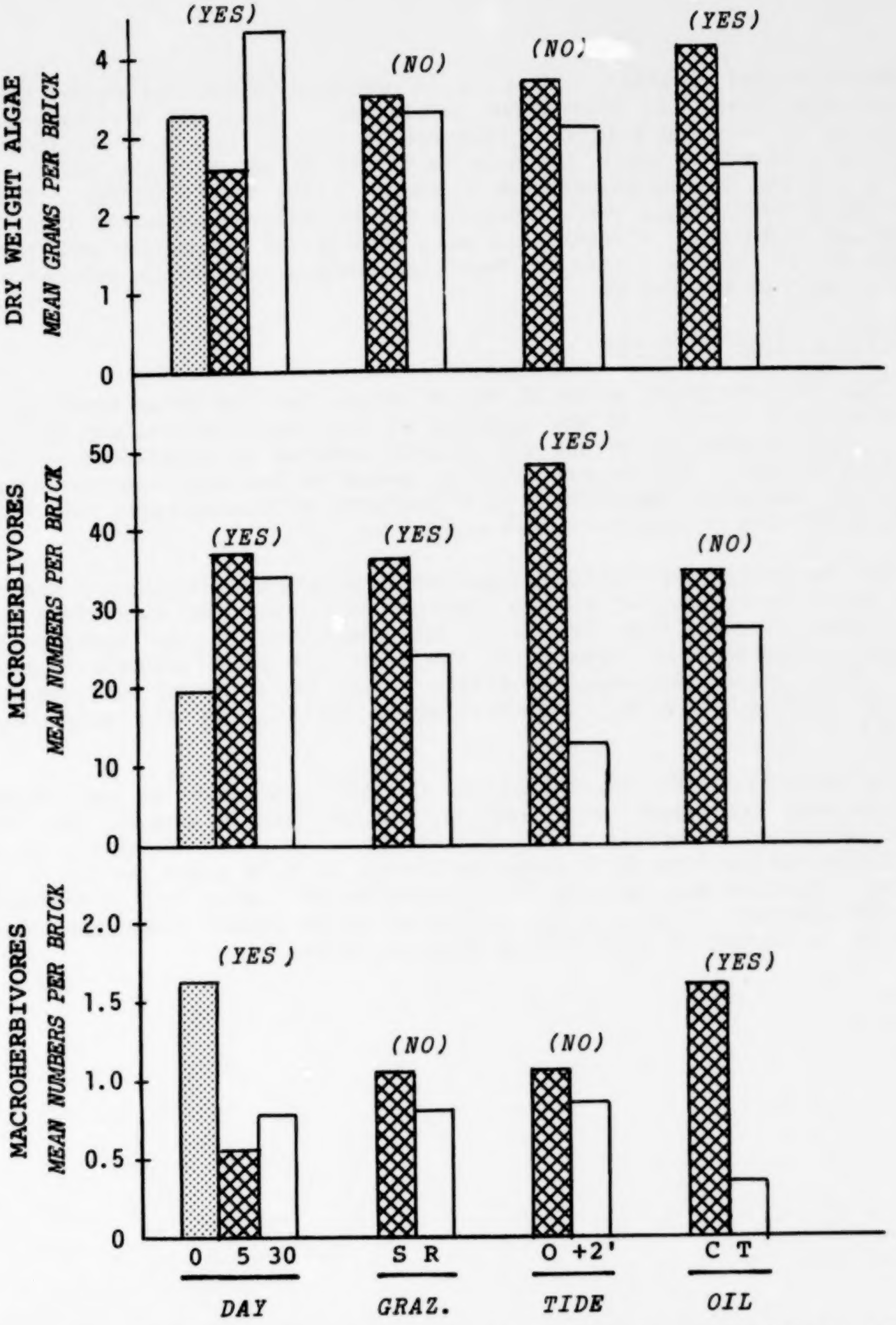
to immediate post-treatment. This is in contrast to the dry weight algal biomass and density of microalgae herbivores. There was a lower mean density on oil-treated bricks as compared to control bricks. Two further points of elaboration about the data in Figure 45 are that the scales for density of the two herbivore groups shown in the figure differ. Thus, there was a much higher overall density for microalgae herbivores than for macroalgae herbivores. Second, the mean density for microalgae herbivores exceeds by at least a factor of four, the numbers of limpets stocked per brick on half of the bricks.

Total Oil on Treated Bricks

Mean concentrations of total oil on bricks for the three experiments are shown in Table 21. It was apparent at the time treatment was applied that a high proportion of the oil present adhered to vegetation on the colonized bricks. This is reflected by comparing the mean concentrations for bricks extracted immediately post-treatment with vegetation intact to bricks which were scraped to remove vegetation.

For the 5-day post-treatment experiment, bricks were scraped to remove algae before extraction of oil and chemical analysis. Mean concentrations were higher at both tide levels at the conclusion of the 5-day post-treatment experiment as compared to the immediate post-treatment experiment. Since no oil was added to bricks in the field, these mean concentrations obviously reflect uncontrolled variability in the extraction methods.

The mean total oil concentrations for the conclusion of the 30-day post-treatment experiment were based on samples from MLLW only, and involved extraction of bricks with algae intact. A substantial reduction in mean concentration from 38.2 grams per brick to 0.79 grams per brick is apparent. Caution must be used in interpreting this as a large difference since the standard deviations are large and, as mentioned above, appear to relate to the amount of vegetation present on bricks.



*NOTE** Different Scales

Figure 45. Mean dry weight algal biomass and density of herbivores of microalgae and macroalgae of experiments at Sequim Bay, May and June, 1980. Main effects of day summarized over all grazers, oil and tides; main effects of grazers summarized over all days, tides and oil; main effects of tide summarized over all days, grazers, and oil; and main effects of oil summarized over all days, grazers and tides. Word in parentheses indicates statistical significance.

Table 21. Total oil concentrations (grams/brick) in grazer experiments at Sequim Bay (May - June 1980).

EXPERIMENTAL CONDITIONS	TOTAL OIL/BRICK (Grams)[1] Mean	S.D.
IMMEDIATELY POST-TREATMENT		
BRICKS SCRAPED TO REMOVE ALGAE		
Top Surface	0.07	(0.09)
Whole Brick	0.25	(0.29)
BRICKS EXTRACTED WITH ALGAE INTACT		
Top Surface	28.45	(23.70)
Whole Brick	38.20	(34.32)
FIVE DAYS POST-TREATMENT		
BRICKS SCRAPED TO REMOVE ALGAE		
MLLW		
Top Surface	0.08	(0.03)
Whole Brick	1.03	(0.34)
+2' ABOVE MLLW		
Top Surface	0.17	(0.04)
Whole Brick	1.32	(0.46)
THIRTY DAYS POST-TREATMENT		
BRICKS EXTRACTED WITH ALGAE		
MLLW		
Top Surface	0.01	(0.00)
Whole Brick	0.79	(0.42)

[1] N = 5 bricks for each mean reported. Standard deviation in parentheses. Total oil measured by IR Spectroscopy.

SECTION 6

DISCUSSION

SIGNIFICANT EFFECTS FROM OIL ON INITIATION OF RECOVERY

These studies have demonstrated statistically significant effects on density and species richness of taxonomic and trophic groups and individual species density due to oil treatment under controlled experimental conditions. An abbreviated summary of these effects is shown in Table 22. From Table 22, 70% of the 56 parameters estimated have been significantly reduced by the oil treatments. In only one case, Mysella tumida, was there an inconsistency in effects shown. For that species, a significantly higher density was shown in oil-treated substrates compared to controls in the sand habitat (Vanderhorst et al., 1980), and a significantly lower density was shown for the species in oil-treated as compared to controls in the Discovery Bay clam bed habitat. The evidence indicates that where significant effects on density were not shown for two of the primary species (Exogone lourei, on the clam bed; and Protothaca staminea, in sand), it was a function of methodological sensitivity and not due to the absence of an effect. In the case of Exogone lourei, the +2' above MLLW tide level was an inappropriate habitat for the species. The highly significant and extreme effect of tide level masked the substantial but smaller effect due to oil treatment. In the case of Protothaca staminea, the density of the species in the sand habitat (Vanderhorst et al., 1980) was far too low for valid comparison.

In addition to the 56 categories listed on Table 22, effects from oil treatment were indicated from descriptive analysis of variance for the density of nearly a third of all other species in the clam bed habitat and for dry weight algal biomass in the colonized epifauna experiments. We believe the evidence is overwhelming that oil treatment, as applied in these studies, is a sharp detriment to the initial stage of recovery in each of the habitats investigated here.

Further perspective concerning the magnitude of the oil treatment effects can be gained by considering those effects in light of other environmental variables investigated.

Season was by far the most important environmental variable for the initiation of recovery based on data in these experiments. For the sand habitat, dependencies in data did not allow us to statistically test for "significant" seasonal differences. However, for experiments conducted in the fall versus ones conducted in the spring, there was an order of magnitude difference in density of primary species and substantial differences in both composition and species richness. The commercial clam bed habitat was investigated in a single season. The densities and richness of taxonomic and trophic groups were significantly affected by month in the

Table 22. Summary of tests for statistically significant biological effects from Prudhoe Bay crude oil treatment.[1]

CONDITION	HABITAT			
	SAND[2]	CLAM BED	BRICK	COLONIZED EPIFAUNA
1. DENSITIES				
POLYCHAETES	-	X	X	X
*_Armandia brevis_	X	X	-	-
*_Exogone lourei_	X	0	-	-
Platynereis bicanaliculata	X	0	-	-
*_Ophiodromus pugettensis_	0	-	-	-
*_Capitella capitata_	0	-	-	-
*_Polydora socialis_	X	0	-	-
CRUSTACEANS	-	X	0	X
*_Leptochelia dubia_	X	X	-	-
*_Photis brevipes_	0	0	-	-
*_Corophium ascherusicum_	X	0	-	-
Exosphaeroma sp.	-	-	0	-
MOLLUSKS	-	X	X	X
*_Mysella tumida_	X	X	-	-
Protothaca staminea	0	X	-	-
Lacuna sp.	0	0	-	-
Transenella tantilla	0	-	-	-
OTHER SPECIES (TAXONOMIC)	-	X	X	X
DETRITIVORES	-	-	0	X
CARNIVORES	-	-	X	X
HERBIVORES	-	-	0	X
SUSPENSION-FEEDERS	-	-	X	X
OTHER SPECIES (TROPHIC)	-	-	-	-
2. SPECIES RICHNESS				
POLYCHAETES	-	X	X	X
CRUSTACEANS	-	0	X	X
MOLLUSKS	-	X	X	X
TOTAL SPECIES	-	X	X	X

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[1] X = Statistically significant effect (P = 0.05). Where * appears, species was _a priori_ selected (P = 0.01).
0 = Statistically significant effect not demonstrated by methods.
- = Parameter not estimated.

[2] Sand Data from Vanderhorst et al. (1970).

seasonally independent experiments conducted in the hard substrate habitat. The range of mean densities over season was often twice (and sometimes three or more times) the range due to oil treatment and tide level. Thus, it can be expected that the magnitude of effect on initial recovery will be strongly affected by season regardless of what perturbation initially removes species from substrates.

Substrate type has a special role in recovery since it can ultimately determine whether a species can survive at all, depending on needs for attachment or burrowing. Our investigation of substrate as an independent experimental variable was inadequate in the present studies. The only statistically valid comparisons made related to effects on primary species densities in sand habitat (Vanderhorst et al., 1980). In those comparisons the texture of the sand (fine versus coarse) resulted in significant effects on density for two primary species, both small bivalves, during the initiation of recovery in the fall (first 3 months). A significant effect due to substrate was not demonstrated for these species after 15 months recovery. The effect of oil treatment was much more severe in those experiments than was substrate. Two other sources of data in these studies bear on the importance of substrate in recovery. The first relates to compositional comparisons between the substrates. We have not formally made these comparisons but it is quite apparent that mollusks were a more important contributor to density and composition on the rock substrate and in the commercial clam bed substrate than they were to sand (Vanderhorst et al., 1980), both in terms of absolute numbers and as a percentage contribution. The other source of data on the importance of substrate in recovery relates to the retention of contaminants and is addressed in the next section.

The importance of tidal height in the initiation of recovery for particular groups is well demonstrated in these studies. For infauna, lower tide heights often resulted in significantly higher densities and species richness. For epifauna, generally higher densities and richness of crustaceans and polychaetes were at lower tide levels, and the reverse was true for mollusks. The effect of the oil treatments on initial recovery of polychaetes and crustaceans in the epifauna was usually slightly less than the effect of tide height difference of two feet. For mollusks, in the epifauna, however, oil treatment effects resulted in higher differences in density and richness than did tide height differences.

We described the geographical location and physical attributes of the four study sites in the methods section. Statistical comparisons of site effect were made between Sequim Bay and Protection Island for infauna recovery and between Sequim Bay and Rocky Point for epifauna recovery. For both habitat types statistically significant effects on density and/or species richness were demonstrated to be due to site. For particular species (*Platynereis bicanaliculata*; *Armandia brevis*), in the infauna experiments reported in Vanderhorst et al. (1980), the effect of oil treatment was as important as the effect of site . For mollusks in the epifauna the effect of oil treatment was more important than the effect of site difference between Rocky Point and Sequim Bay. Because of untenable assumptions, statistically valid comparisons of site effects between Sequim Bay and Discovery Bay were not possible in these studies. However, mean data on species richness (Figure 27) and taxonomic and trophic group densities (Figure 28) showed remarkable similarities between the two sites.

OIL TREATMENT AND RETENTION OF OIL IN EXPERIMENTS

Total oil concentration at the initiation of experiments with infauna ranged from a low of about 1,000 ppm for the Sequim Bay summer experiment (Vanderhorst et al., 1980) to a high of about 2,500 ppm total oil in the commercial clam bed experiment. Fall and long-term experiments at Sequim Bay (Vanderhorst et al., 1980) were intermediate in initial concentration with about 1,800 ppm total oil. Although the mixing of oil in sediment does not occur in every oil spill incident, the total amount of oil in experimental sediments, even in the highest concentration case (2,500 ppm), was no more than 25% of the approximate 10,000 ppm measured in sediments following the AMMOCO CADIZ spill. There was one anomaly in the analyzed saturate and aromatic compound data noted for the summer experiment at Sequim Bay in that the initial concentration of analyzed aromatic compounds was quite low as compared to other experiments. The experimental designs were such that we cannot attribute differences in biological effects to differences in the initial treatment concentration of oil. In each set of experiments, the oil treatment *per se*, as documented and verified by a large number of measurements of *total* oil and saturate and aromatic compounds, was evaluated as a two-level factor (treated or untreated).

The distribution of oil in time and space within the experimental designs could be expected to relate to the pattern of faunal recovery in time and space. The most striking difference in oil retention by sediments was the difference between Sequim Bay sediments and those from the commercial clam bed on Discovery Bay in 3-month summer experiments. For the former case (Vanderhorst et al., 1980), initial oil concentration was reduced by 48% on the average; while for the commercial clam bed experiments losses amounted to only 12.5% on the average. This sort of a difference is of great interest because, based on the evidence we have presented, it will directly affect the initiation of recovery.

A part of this differential in retention can be explained by tidal height differences. In the summer experiment at Sequim Bay, the -2' tide level had 47% of initial concentration; the MLLW tide level had 57% of initial concentration. At Discovery Bay, the MLLW tide level had 80% of initial concentration and the +2' tide level had 95% of initial concentration.

At least three other factors may contribute to the difference in retention seen in these studies: (1) wave exposure; (2) initial concentration; and (3) sediment texture and organic content. Wave exposure was not measured in these studies, but we judge that for the period of the experiment, the two sites were roughly equivalent and received moderate wave activity for inland waters. In terms of initial concentration, the concentration at Discovery Bay was nearly 2.5 times as high as the concentration at Sequim Bay. We have MLLW data showing a consistent trend of percentage retention to initial concentration in these studies; however, the data are confounded by season, and statistical comparison is inappropriate. At Discovery Bay there was an initial concentration of about 2,500 ppm and a retention of 80% in three months; at Sequim Bay, in a fall 1978 experiment of three months duration, there was an initial concentration of 1,758 ppm and a retention of 66%; in the Sequim Bay summer experiment, as stated above, there was an initial concentration of 1,069 ppm and a retention of 57%. The thesis behind this observed trend could, of course, be that the more oil present, the greater the proportion retained.

It appears that tide level and initial concentration factors adequately account for the differences in proportional retention of oil seen in these studies. However, very clear differences in sediment retention time for oil have been shown by other investigators relating to sediment particle size and organic content, with smaller grain size retaining oil much longer than coarser ones. Thus, a smaller grain size dominance at Discovery Bay would be consistent with the longer retention times seen in these studies. In fact, from the limited data presented on grain size patterns from experimental sediments in the present studies , the grain size patterns at the two sites differ chiefly in the higher contribution of a gravel (greater than 5.66 mm diameter) phase at Discovery Bay.

From the Sequim Bay/Protection Island data on the sand habitat (Vanderhorst et al., 1980), we were able to develop a model of oil loss from sediments to predict a background level of total oil in 18.5 months. The model was developed from experimental data which showed a high initial rate of loss followed by a much lower rate of loss thereafter. The lower rate was fairly stable between three and 15 months after initial contamination. If that lower rate (71 ppm per month) is applied to concentrations of total oil remaining at Discovery Bay at the end of three months, then complete depuration of those sediments would occur by the end of 31 months. From the available data it is impossible to judge if this is a liberal or conservative estimate. Further experimentation is warranted.

Data from capillary gas chromatography confirm the general trends in retention time established by total oil concentrations above. One very distinct difference between Sequim Bay experiments and the Discovery Bay experiment relates to analyzed aromatic compounds. In both summer and fall experiments at Sequim Bay, the loss of analyzed aromatics was proportionally much more rapid than the loss of saturates. Only 14% of analyzed aromatics remained in the Sequim Bay fall three-month experiment. In the summer experiment, analyzed aromatics were at background levels in three months (Vanderhorst et al., 1980). In sharp contrast, analyzed aromatic concentrations were unchanged from initial concentrations in the Discovery Bay experiment after 3 months of field exposure.

In addition to the differences in retention time due to tide level, sediment types, and initial concentration noted above, the experimental approach also differentiated the effect of sediment depth on oil retention. This factor is important to recovery since larval or young organisms settle in surface sediments, and penetrate deeper into sediments as they grow. Our approach involved a simple separation of the top and bottom halves of cores. Core length was equivalent to tray depth (15 cm). At the end of three months there were significantly higher concentrations of total oil and analyzed aromatic compounds in the bottom half of cores as compared to the top. Based on the three-month time frame of the present experiment, this significant differential in concentration probably had little effect on observed recovery. We base this thesis on the fact that the same general magnitude of effects were seen in Sequim Bay experiments where total oil concentration in whole cores was less than half that seen in the Discovery Bay experiment. The longer term implication to recovery may, however, be great. Oil deep in sediment strata degrades more slowly than in the upper strata because of less oxygen and mechanical action (Westlake, 1980).

The oil treatment characterizations used for hard substrates do not relate easily to real-world spill situations; and, thus, there is heavy reliance on the method of application in making judgments about the severity of treatment in these studies. We do have data on the partitioning of oil in these experiments which aid in making such judgments. The chemical characterization of substrates provides insight into the effect of tide level, site, and duration on the retention of oil within the experiment itself. The top surface, whole brick partitioning in the extraction procedures provides a basis for best case and worst case retention of oil.

Both total oil and analyzed saturate and aromatic compound classes showed a rapid loss of oil from the top surface of bricks. Only 16% of total oil concentration on the top surface remained five days after treatment (computed from Table 11). A further reduction to 12% of the initial concentration at the 30-day post-treatment period was only slight and not significantly different from five-day concentrations in spite of the fact that 50 replicate bricks were used in each part of the comparison. Coupled with the fact that the amount of biological colonization in this same five-day period was, over all experiments, negligible as compared to 30-day colonization, we infer that effects attributable to the oil treatment were due to rather small amounts of oil (less than one gram per brick). It is also pertinent, from Figure 20, that excessively high concentrations of oil immediately post-treatment bear no relation to five-day top surface concentrations. Thus, if the treatments applied initially to bricks (except for the grazer experiments involving colonized bricks) were unrealistically severe, the actual amounts of oil to which colonizing organisms were exposed, were not.

The whole brick extractions of oil indicated that a reservoir of oil may exist in the pores of the brick substrate for extended periods. For the total oil concentrations, this amounted to 49% of initial concentration for five days and 34% of initial concentration for 30 days. We have no evidence that this reservoir influences the top or biological exposure surface concentration. This characteristic of retention is, of course, unique to the substrates used. While it would probably be a safe assumption that the concrete bricks used in these experiments fall within the range of porosity of natural substrates of the Strait of Juan de Fuca region, we have no evidence concerning this.

The tide level distribution of oil retained on bricks over one-month periods is defined by total oil data which indicate a slightly greater retention of oil at the +2' tide level as compared to MLLW. The analyzed saturate and aromatic compound data showed the reverse of this tide level trend at 30 days post-treatment. However, the replication used for obtaining those means was limited to five bricks per condition (a single month) and the month chosen for these detailed analyses was atypical.

There were not significant differences in total oil concentration for bricks related to the site of experimentation between Sequim Bay and Rocky Point.

Using the total oil data on Table 11 as a base for calculation, and the rate of loss between five and 30 days as a rate which may be representative of longer term losses, we calculated a top surface background concentration would occur in 3.73 months from the termination of treatment, and a whole brick background concentration would occur in 2.93 months. Obviously, the rate of loss for whole bricks is at the end of 30 days a much higher rate (grams per brick) than the top surface rate. This whole brick calculation can be taken as a "best" case. If we use rate of loss seen for the top surface of bricks to compute loss of the amount of oil remaining in the whole brick at the end of 30 days, it is much slower than would be expected, and represents, perhaps, a "worst" case for retention of oil. The calculation predicts a total loss of oil from bricks in 13.5 months. Thus, even using the worst case, the retention of oil on hard substrates is of substantially shorter duration than for the sand and mud substrates (18.5 and 31 months) discussed previously.

Although the retention and distributon of total oil in the grazer manipulation experiments did not differ substantially from the patterns shown for other hard substrate experiments, it should be noted that since the preponderance of organisms were present on bricks at the time the treatments were applied, the severity of the treatments relative to the organisms measured was tremendously greater (orders of magnitude in terms of oil concentration).

IMPLICATIONS OF THE OIL TREATMENTS FOR OVERALL RECOVERY

The implications to overall recovery from the effects of oil discussed previously differs depending on the habitats and specific task objectives.

Perhaps the simplest case relates to the infauna community in the sand habitats which has been previously discussed (Vanderhorst et al., 1980). In that case the 15-month recovery experiments were of sufficient duration for control substrates to gain full recovery in terms of individual species and aggregate density, and species richness. Thus, comparisons derived directly from the experiments allowed expression of effects on recovery in terms of densities and species richness as a percentage of the fully recovered controls. In that case, species richness was essentially recovered in 15 months while individual and aggregate densities lagged control densities by 50% at the end of 15 months. By using data on oil retention in those studies, an oil-free habitat could be predicted to occur 18.5 months after the initial treatment of about 1,800 ppm total oil. By adding the time to predicted total oil loss and demonstrated time to recovery in controls (15 months) we arrived at a total recovery time of 33.5 months. In that particular set of experiments, initiated in late summer, and terminated in November the following year, the only extrapolation involved, i.e., a further 3.5 months for total oil loss, was during a winter low recruitment period. It is probably not critical if the extrapolation is not entirely accurate. The rationale for predicting recovery assumes that a total depuration of oil from sediments is required before full recovery can be realized. That assumption has not been tested in these studies. However, partial recovery can occur even when substantial amounts of oil remain in sediments as indicated by the compositions and densities in the oil-treated sediments. Thus, our estimates are worst case for the experimental conditions used.

The overall similarity in species richness and density between the Sequim Bay site and the Discovery Bay commercial clam bed site controls at the end of three months, shown in Figures 17 and 28, suggests to us that the "fully recovered" condition in 15-month controls for Sequim Bay experiments will also fairly represent conditions for controls at Discovery Bay. Thus, using the same worst case computations as applied above and the longer oil retention time estimates for Discovery Bay (31 months), we predict a full recovery from the 2,500 ppm oil treatment in 46 months (31 + 15 = 46). Obviously, because of the necessary extrapolation of rate of loss of oil from sediments beyond the experimental time frame, there is considerably less assurance in this estimate than for the Sequim Bay habitat.

The principal focus in the commercial clam bed experiment was the effect of oil on recovery of the littleneck clam (Protothaca staminea). Fortunately, 1980 was a typical settlement season for the species in Discovery Bay. A statistically significant effect from oil on the density of this species was demonstrated. From the mean density data presented in Table 15, the magnitude of mean difference is much greater at the MLLW tide level than at the +2' tide level. We reviewed the data for individual cores and trays to assure ourselves that this mean difference was not attributable to high or low density in one or a few cores, or a single tray. This was not the case. For this particular species, the demonstrated effect is an especially conservative estimate since the recovery period covered only about half of the species' normal recruitment period and the concentrations of oil were still extremely high at the termination of this experiment.

We presented data indicating a vertical stratification of retained oil concentration after three months in the commercial clam bed habitat. These data suggest that the surface sediments where young clams initially settle may be free of oil at an earlier time than the projected 31 months; while sediments at greater depth, but within 15 cm of the surface, may retain oil for appreciably longer periods. The effects of this oil on larger clams which penetrate deeper into the sediment has not been investigated in these studies.

The significant effects on organisms colonizing bricks in the month-long experiments with hard substrates clearly establish an important role that oil plays in the initiation of recovery processes. The duration of these experiments represents such a small proportion of the time reportedly required for development of a "mature" rocky intertidal community that meaningful recovery rate predictions are out of reach. Two prevailing views of oil pollution effects on recovery in the rocky intertidal are at odds.

The first view, subscribed to in our region by Nyblade (1979), holds that the rocky intertidal communities consist chiefly of long-lived organisms with infrequent successful recruitment. From this view our experimental design has a demonstrated relevance. Clearly, oil, as applied in these experiments, interferes with the initiation of the recovery process. The end result of these recovery processes may require decades. Unfortunately, despite decades of imaginative experimental research and biological survey, quite apart from oil pollution research, there is not a clear single definition of a fully recovered rocky intertidal community (there are many). Indeed, a demonstration of the relative importance of only a few of the many dependencies inherent in a fully recovered concept is only beginning. Among all studies of the rocky shore, catastrophic physical processes, principally from wave action, play a large role in restructuring communities by completely denuding sections of the shore. The second view of the role of oil in the recovery of the rocky intertidal assigns oil as just another catastrophic event in many. Thus, the effect of oil in recovery lasts only so long as the oil is present. In concert with the second view, our data indicate that while the preponderance of oil may be washed from substrates isolated in a large matrix of clean substrate and oil-free sea water within a period of five days, a small residual amount adheres to the substrate. Within a month-long time frame this residual amount is sufficient to produce significant effects on recovery. The "best" and "worst" cases for total loss of this oil are approximately three and 13 months.

The grazer manipulation studies identify the potential of oil treatment to reduce species richness and density, and algal biomass in existing rocky shore communities. The effect of experimentally shifting the balance in grazer density was shown to significantly increase mollusk and herbivore densities, groups to which the manipulated species belonged, and to significantly decrease the density of polychaetes. Within the 30-day time frame of the experiments, the oil treatment resulted in a significant decrease in algal biomass and the experimental stocking and removal of algal grazers did not. The absence of an effect from grazer manipulation undoubtedly relates in part to the fact that the principal algal biomass was macroalgae while the grazers manipulated were feeders on microalgae.

During the 30 days post-treatment, in the grazer experiments overall, there were significant increases in density for crustaceans, mollusks, herbivores, and suspension-feeders, and significant increases in species richness for polychaetes. There was a significant increase in algal dry weight biomass. The studies were not of long enough duration to evaluate the possible effects from upsetting the structure in these communities. The loss of oil from the colonized substrates proceeded at a rate comparable to the rate obtained for monthly experiments previously discussed.

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